Prostaglandins in ovulation

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Summary. Evidence accumulated using prostaglandin synthesis inhibitors, prostaglandin measurements, or prostaglandin antibodies in several species has supported the concept that a gonadotropin-induced preovulatory rise in follicular prostaglandins is required for ovulation. There are at least three questions which need to be answered for a further understanding of this requirement : 1) What is the mechanism by which gonadotropin can stimulate prostaglandin synthesis ? 2) In which cell type(s) are prostaglandins produced, and upon which do they act ? 3) What are the actions of prostaglandins which participate in ovulation ? There have been recent advances which partially answer the first two questions. Gonadotropins appear to stimulate prostaglandin synthesis by increasing the quantities of follicular cyclic AMP (or possibly cyclic GMP). Derivatives of both nucleotides have been found to be effective exogenously. The effect of gonadotropin on prostaglandin synthesis also exhibits a delay of several hours and requires macromolecular synthesis. As to the first portion of question two, it appears that granulosa cells produce the bulk of follicular prostaglandins. It is not clear at this time, however, which cell(s) respond to the preovulatory rise in follicular prostaglandins, nor precisely how that response relates to ovulation.

Introduction.

Considerable evidence exists supporting a role for prostaglandins in the process of ovulation in the rat and rabbit (LeMaire and Marsh, 1977). Inhibition of prostaglandin synthesis by the systemic or local administration of indomethacin or aspirin has been shown to block ovulation in the rabbit and the rat (Behrman and Caldwell, 1974 ; Zor and Lamprecht, 1977). Since this block could not be overcome by LH, but could be reversed by administration of exogenous prostaglandins, the prostaglandin involvement appeared to be at the ovarian level. Earlier, it had been shown that rabbit ovarian follicles undergo marked changes in prostaglandins around the time of ovulation (fig. 1 ; Armstrong *et al.*, 1974 ; Bowring *et al.*, 1975). Prostaglandins of the F (PGF) and E (PGE) series both increased in ovulated follicles, but not in follicles which failed to ovulate (fig. 1). These increases were not seen when indomethacin was administered systemically (Yang *et al.*, 1973 ; Armstrong *et al.*, 1974) or intrafollicularly (Armstrong *et al.*, 1974). In addition, intrafollicular injection of a prostaglandin antibody prevented LH-induced ovulation only in the injected follicle (Armstrong *et al.*,

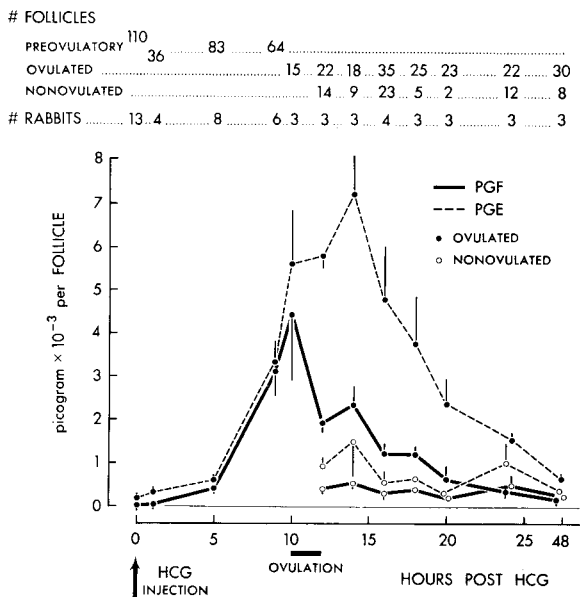1974). Antiserum to PGF was more effective than that to PGE in these experiments in rabbits.

FOLLICLES

FIG. 1. — *Pre- and postovulatory changes in prostaglandin levels in rabbit Graafian follicles.* The ovulatory process was initiated by the intravenous injection of 100 i.u. of hCG. At 5 and 9 hr the large preovulatory follicles were dissected and radioimmunoassay of follicular PGE and PGF carried out. At 10 hr and later the follicles that had ovulated by morphological criteria were measured as the group of ovulated follicles. From 12 hr onward, the large follicles which did not show evidence of ovulation were also dissected and grouped as nonovulated follicles. The number of each type of follicle analyzed and the number of animals at each time are indicated at the top of the figure. The points represent the mean, and the brackets the standard error of the mean. When no brackets are shown, the point represents the average of only two determinations (From Yang *et al.*, 1974).

A similar increase in prostaglandins in preovulatory follicles has also been observed in adult rats (LeMaire *et al.*, 1975 ; Bauminger and Lindner, 1975) and in immature rats treated with PMSG (Armstrong *et al.*, 1974 ; Iesaka *et al.*, 1975). Prostaglandins increased after either an endogenous surge of LH or administration of gonadotropin.

Although not as extensively studied as the rat and rabbit, there is some evidence that prostaglandins are involved in ovulation in many other species. Inhibition of prostaglandin synthesis blocked ovulation in the goldfish, mouse, and monkey (LeMaire and Marsh, 1977). Preovulatory increases in prostaglandins have been reported in swine (Ainsworth *et al.*, 1975). Stimulation of ovarian prostaglandin synthesis by LH *in vitro* has been observed for follicular tissue from bovine (Shemesh and Hansel, 1975) and human (Plunkett *et al.*, 1975) origin.

Not all of the available data, however, supports a role for prostaglandins in ovulation. In at least one species, the chicken, administration of indomethacin (in a dose sufficient to significantly inhibit PGF levels in preovulatory follicles) failed to

block ovulation (Day and Nalbandov, 1977). The administration of aspirin to women (Chaudhuri and Elder, 1976) and active immunization against PGE_2 and $PGF_{2\alpha}$ in rats (Bauminger, 1977) also failed to block ovulation, although it was not determined in these studies if in fact follicular prostaglandin increases were inhibited. With these possible exceptions, then, prostaglandins have been shown to have an important role in the process of ovulation.

Current studies

Following the earlier observations made *in vivo*, certain aspects of follicular prosta-glandin synthesis were investigated *in vitro* by ourselves and others.

One such aspect was the role of cyclic AMP in LH stimulation of prostaglandin synthesis. Graafian follicles isolated from estrous rabbits and incubated for 5 hrs with LH (5 μg/ml) produced increased quantities of both PGE and PGF (Marsh *et al.*, 1974). PGF synthesis in rabbit follicles was also stimulated by LH in an organ culture system (Moon *et al.*, 1974). This effect was specific for LH and could not be elicited in rabbit follicles by FSH, prolactin, or BSA (Marsh *et al.*, 1974). The addition of cyclic AMP (cAMP), however, could mimic the action of LH (fig. 2).
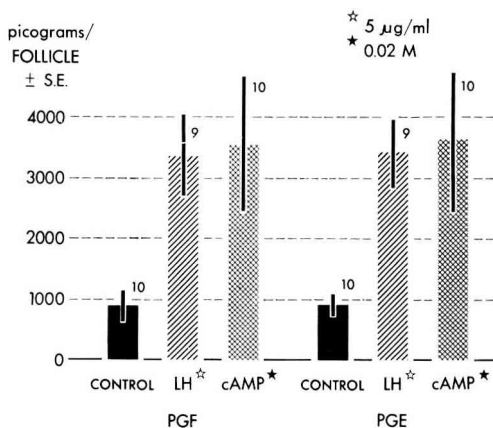

FIG. 2. — *Prostaglandin synthesis in isolated rabbit Graafian follicles incubated* in vitro : *the effect of LH and cyclic AMP*. Follicles were isolated from the ovaries of estrous rabbits and incubated indivi-dually in buffer alone (control), or with the addition of LH or cyclic AMP (cAMP). Prostaglandins were measured by radioimmunoassay. The height of the bars represents the mean prostaglandin synthesis and the vertical lines depict the standard error of the mean. The numbers above the bars indicate the number of follicles in each group (From Marsh *et al.*, 1974).

The mediation of LH stimulation of PGE synthesis by cyclic AMP was studied further with isolated rat Graafian follicles. It was found that ATP, ADP, 3′-AMP, 5′-AMP, and cyclic GMP were not capable of stimulating PGE synthesis, while cyclic AMP and 1-methyl-3-isobutylxanthine produced significant stimulations (Clark *et al.*, 1978). Derivatives of cyclic AMP such as dbcAMP, N^6-mbcAMP, and 8-BrcAMP, were also effective (Clark *et al.*, 1978). In contrast, it has been reported that 8-BrcGMP can stimulate PGE production in rat follicles, although conclusive data on the effect of

LH on follicular cyclic GMP is not available (Zor et al., 1977). In addition, it has been shown that cholera toxin increases both cAMP and PGE in rat follicles (Clark et al., 1978).

Since cyclic AMP is also known to increase ovarian steroidogenesis, the possibility existed that the action of LH on prostaglandins was mediated by this effect on steroidogenesis (LeMaire and Marsh, 1975). This proposal was disproven by the finding that the inhibition of steroidogenesis by aminoglutethimide did not prevent the LH-induced rise in prostaglandins (Bauminger et al., 1975).

It should be noted that a time lag of 3 hrs or more was observed in vivo (fig. 1 ; Bauminger and Lindner, 1975) and in vitro (Clark et al., 1976) between exposure of follicles to LH and detectable increases in prostaglandin synthesis. This lag did not appear to be due to a requirement of sufficient time for increased steroidogenesis to occur, as just discussed.

Since cyclic AMP could cause an increase in prostaglandins in rabbit follicles (Marsh et al., 1974), it could be postulated that the time lag was due to a delay in production or action of cyclic AMP. There does not appear to be a time lag, however, in production of cyclic AMP by rat follicles (Nilsson et al., 1974). Instead, the delay seems to be in the expression of the action of cyclic AMP, since exogenous cyclic AMP produced a delay similar to LH in incubated rat follicles (Clark et al., 1978). A similar lag was also observed in the stimulation of PGE production by 8-Br-cyclic GMP in rat follicles (Zor et al., 1977).

The latent period, on the other hand, supported a requirement for macromolecular synthesis as a prerequisite to increased prostaglandin synthesis (Bauminger and Lindner, 1975). Such a requirement was found using isolated follicles from PMSG-treated immature rats as a model system. When Graafian follicles from these animals were incubated with LH, a marked increase in PGE synthesis was observed after 5 hrs (fig. 3). This increase was blocked by simultaneous incubation with 10 μM
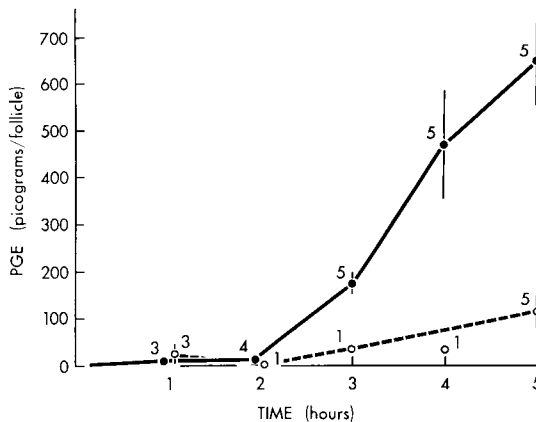

FIG. 3. — *Time course of prostaglandin accumulation by rat follicles* in vitro : *effect of LH.* Preovulatory follicles were isolated from PMSG-treated immature rats and preincubated for 1 hr. Groups of 3 follicles were then further incubated in fresh KRBB in the presence (o—o) or absence (o— —-o) of 5 μg/ml of LH (NIH-LH-S18). The total accumulation of PGE was measured by radioimmunoassay. The number of experiments appears adjacent to each point on the graph. Data are expressed as mean ± standard error (From Clark et al., 1976).

puromycin, and not by 5 μM puromycin aminonucleoside (Clark et al., 1976). Actino-mycin D (5 μM) and cycloheximide (10 μM) were also capable of inhibiting the effect of LH (Clark, unpublished). The step in LH action requiring macromolecular synthesis appears to be beyond the production of cyclic nucleotides, since the effect of 8-BrcAMP (Clark, unpublished) and 8-BrcGMP (Zor et al., 1977) can also be blocked by cyclo-heximide. In this regard, an increase in follicular protein and RNA synthesis was found to precede ovulation and this increase was probably induced by the preovulatory gonadotropin surge (Mills, 1975). Furthermore, the direct intrafollicular injection of inhibitors of RNA and protein synthesis is capable of blocking ovulation in the rabbit (Pool and Lipner, 1966). The nature of the requirement for macromolecular synthesis, however, remains unknown.

For the further study of the role of prostaglandin synthesis in ovulation it is important to define which cell type(s) in the follicle produces prostaglandins. Current data suggest that granulosa cells are the source of prostaglandins. The separate culture of theca and granulosa cells from follicles of estrous rabbits for two days showed the majority of the PGF to be present in granulosa cells (Erickson et al., 1977). PGE was not measured in this study. In our laboratory, however, it has been observed using acute 5 hr incubation of separated components from preovulatory rabbit follicles, that the majority of the PGE is also produced by granulosa cells (Triebwasser, unpublished). It appears, therefore, that the granulosa cells are responsible for most or all of the production of PGE and PGF in the rabbit follicle. These findings allow the development of more definitive approaches to the regulation of prostaglandin synthesis by utilizing a single cell type.

Model for the role of prostaglandins in ovulation

Figure 4 attempts to summarize the position of prostaglandins in the scheme of events leading to ovulation (LeMaire et al., 1977). It is not assumed that all of the events depicted occur in a single cell type, although many of them are capable of occuring in isolated granulosa cells. The ovulatory surge of LH initially acts upon a follicular cell by binding to its receptor, activating adenyl cyclase, and causing a rise in intracellular cyclic AMP. In the rat, FSH may also play some role in this process (LeMaire at al., 1977). The steps which follow the rise in cyclic AMP are only partially understood. They appear to involve protein kinase activation, protein synthesis, increased steroidogenesis, and prostaglandin synthesis. The manner in which prostaglandin accumulation is regulated could be by substrate availability, or by the amount of prostaglandin synthetase, or by changes in prostaglandin conver-sion to inactive metabolites. A clear choice between these alternatives cannot be made at this time (LeMaire et al., 1977).

The function of the preovulatory prostaglandin increase in the ovulatory process remains to be discovered. It has been suggested that follicular rupture involves an enzymatic weakening of the follicular wall (Bjersing, 1977), and prostaglandins might be involved in the activation, release, or synthesis of an ovulatory enzyme. Prostaglandins could also possibly act by influencing smooth muscle contraction, which has a proposed role in ovulation (Wallach et al., 1977). Recent observations, however, have not supported an effect of prostaglandins on contractility, since indome-
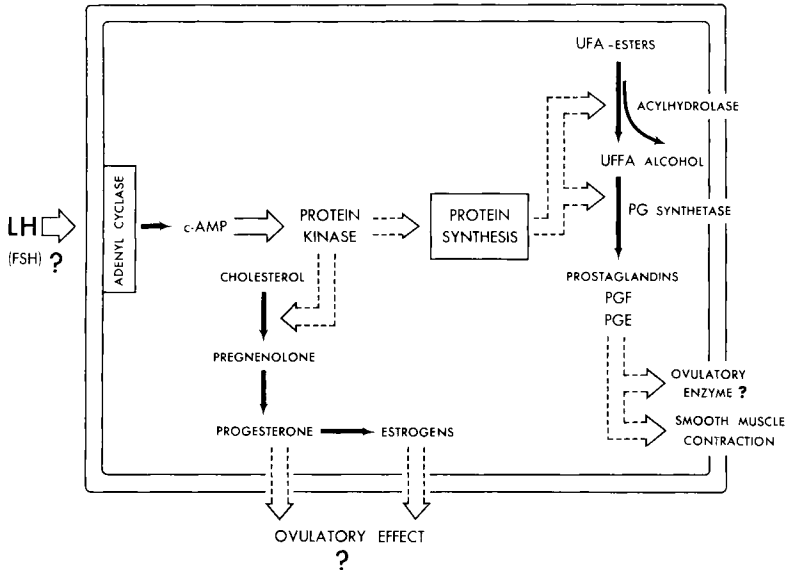
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FIG. 4. — *Hypothetical model of some aspects of the biochemical events in the ovulatory process. Abbreviations used are : LH = luteinizing hormone ; FSH = follicle stimulating hormone ; cAMP = cyclic AMP ; UFA = unsaturated fatty acids ; UFFA = unsaturated free fatty acids ; alcohols = lysophospholipids, cholesterol and diglycerides. Solid arrows indicate biochemical conversions. The open and dashed arrows indicate stimulation. The dashed arrows imply possible, but yet unproven actions (From LeMaire et al., 1977).*

thacin-induced reduction in ovulation in perfused rabbit ovaries was not accompanied by changes in ovarian contractility (Hamada *et al.*, 1977). The function of prostaglandins in ovulation, therefore, remains obscure and must await further definition of the process of ovulation on a morphological and biochemical basis.

Résumé. Une augmentation préovulatoire des prostaglandines dans les follicules, induite par la gonadotropine, semble être nécessaire pour l'ovulation. Cette hypothèse s'appuie sur les résultats des études dans plusieurs espèces des prostaglandines dans les follicules, en employant des inhibiteurs de prostaglandines ou des anticorps contre les prostaglan-

dines. Au moins trois questions demandent des études approfondies pour comprendre le rôle des prostaglandines : 1) Par quel mécanisme la gonadotropine est-elle capable de stimuler la synthèse des prostaglandines ? 2) Dans quel(s) type(s) de cellules les prostaglandines sont-elles synthétisées et sur quel(s) type(s) de cellules peuvent-elles agir ? 3) Quelles sont les actions des prostaglandines participant au mécanisme de l'ovulation ?

Des résultats d'expériences récentes peuvent partiellement résoudre les deux premières questions. Notamment il a été démontré que la gonadotropine stimule la synthèse des prostaglandines par l'intermédiaire d'une augmentation de l'AMP cyclique dans les follicules (probablement aussi du GMP cyclique). C'est-à-dire que l'addition de dérivés de ces deux nucléotides est capable de stimuler la synthèse des prostaglandines dans les follicules isolés et incubés in vitro. Il a été également trouvé que l'action stimulante des gonadotropines sur la synthèse des prostaglandines se manifeste après un délai de plusieurs heures, nécessitant la synthèse de macromolécules.

Finalement, les cellules de la granulosa semblent produire la majorité des prostaglandines folliculaires.

Quelles sont les cellules influencées par l'augmentation des prostaglandines et comment cette augmentation agit dans le mécanisme de l'ovulation restent actuellement presque complètement inconnus.

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