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COMMENTARY

ROLE OF ANGIOTENSIN II IN THE PROCESSES LEADING TO OVULATION

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RENIN–ANGIOTENSIN SYSTEM

The renin–angiotensin system (RAS) is a mixed enzymatic–hormonal system. Renin is the first enzyme in the biochemical cascade of RAS. Up to 90% of human plasma renin exists in plasma in the inactive form which seems to be the biosynthetic precursor of the active enzyme. The mechanism of prorenin activation has not been elucidated completely. Enzymatically active renin cleaves its substrate, angiotensinogen, to form angiotensin I (AI). This decapeptide is, in turn, converted to angiotensin II (AII) by angiotensin-converting enzyme (ACE). AII, the main active component of RAS, is a potent vasoconstrictor and a primary stimulus for aldosterone biosynthesis [1]. This confers upon RAS the ability to exert its functions in the maintenance of blood pressure and electrolyte balance as well as fluid homeostasis.

A number of local RASs have been described in tissues such as blood vessel walls [2], kidney and adrenal [3, 4], brain [5] and the reproductive tract [3, 4, 6], raising the possibility of autonomous intrinsic RASs in such tissues. The result could be local regulatory mechanisms unique to each individual tissue source. Recently, the evidence for an active RAS in the ovary [7] has raised the possibility of an action for AII in follicle development and ovulation.

OVARIAN RENIN–ANGIOTENSIN SYSTEM (OVRAS)

The identification of renin activity [8] and AII immunoreactivity in follicular fluid [9] raised the possibility that AII generated within the ovary may have localized effects. In this article, we will review and discuss the localization, function and possible roles of AII in the ovary. We will follow events and processes which indicate that AII may be part of an intrinsic ovarian regulatory system that assists in the orchestration and synchronization of the ovulatory process (Fig. 1).

Early follicular development

(a) Theca dominance. The earliest phase of follicular growth is characterized by an increase in follicular diameter in response to proliferation, differentiation and early function of activated granulosa and theca cells. During this stage, angiotensin has been localized by immunohistochemistry to the theca cells [10]. *In vitro* autoradiographic analysis of rat ovarian sections has shown AII receptors over metestrus and diestrus follicles [11] which have been discretely localized to the granulosa and theca cells of developing follicles [12, 13]. Finally, human thecal cells in culture have been shown to generate renin [14].

The proliferating theca cells become highly vascularized, suggesting involvement of AII in the induction of neovascularization accompanying folliculogenesis. Fernandez *et al.* [15], using a rabbit cornea model, demonstrated that AII has angiogenic properties. Frederick *et al.* [16, 17] injected purified fractions from human and porcine follicular fluid into rabbit cornea pockets and showed striking angiogenesis. Thus, evidence suggests that follicles, as well as corpora lutea, produce angiogenic factor(s) [18]. At least part of this activity appears to be associated with a heat labile basic peptide whose stimulus for action is enhanced by gonadotrophin treatment [19]. The source of this ovarian angiogenic activity is controversial and not clear; the follicular granulosa or theca cells seem likely, but not exclusive sites of synthesis. In one of the reports by Frederick *et al.* [17], porcine angiogenic activity was demonstrated in two distinct molecular weight ranges: 45,000–60,000 and 1500 daltons. These findings are consistent with low molecular weight angiogenic factors such as AII and AIII which are octa- and septapeptides, respectively, perhaps combined with higher molecular weight carrier proteins. In any case, a recent report by Sterling *et al.* [20] indicates that AII induces the mRNA for the angiogenic factor, basic fibroblast growth factor. Thus, AII may indeed participate in neovascularization.

It has also been shown that access *in vivo* to hormones or other trophic factors and nutrients may be limited to differences in the vascular supply to individual follicles [21]. Angiotensin, being one of the most potent vasoactive substances, may take part in the shunting of blood within the ovary.

The theca early on contains LH receptors and responds by secreting androgens [22]. These androgens can reach the granulosa cell layer through the porous basement membrane of the follicle. In addition to serving as precursors for the estrogen produced by the aromatase of the granulosa cells [23], androgens act as powerful anti-estrogens by inhibiting estrogen receptor formation [24]. Thus,

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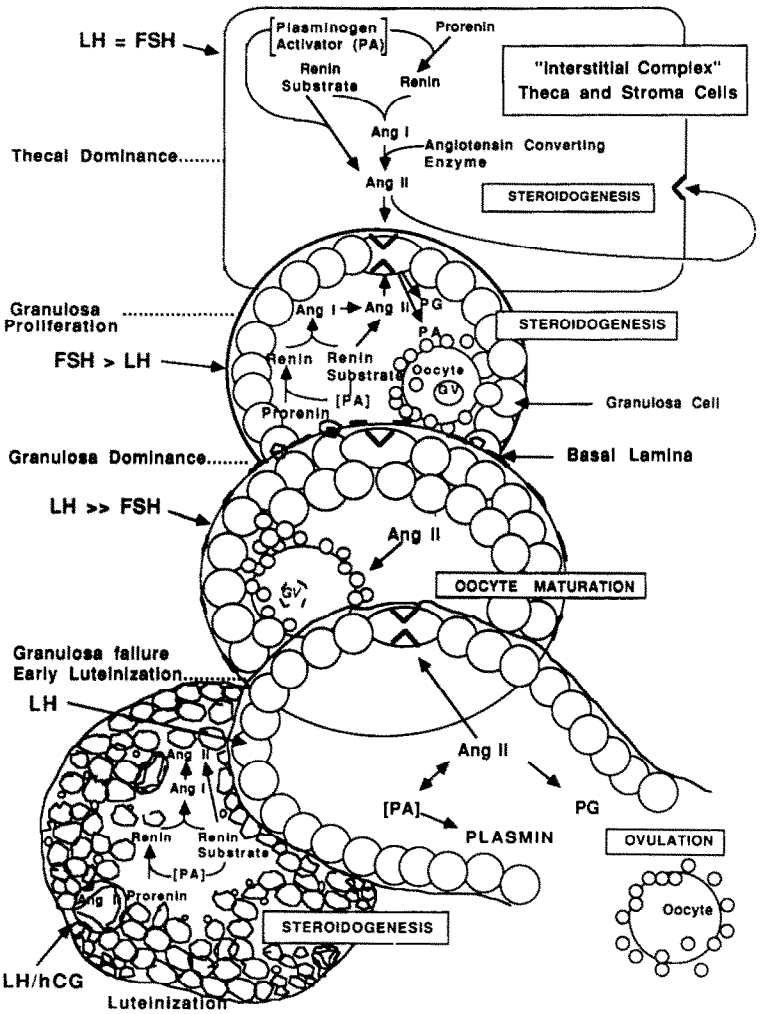

Fig. 1. Stages of ovarian compartmental development (from Palumbo *et al.*, [10]).

the early development furnishes primarily thecal androgens whose fate is decided by the quantitative (aromatization to estrogens) and qualitative (anti-estrogen action of androgens) characteristic of the granulosa layer. This can be androgen-driven follicular atresia [25] or estrogen-driven granulosa cell proliferation and steroidogenesis [26]. Evidence from bovine follicular fluids indicates that atretic follicles (i.e. follicles with high progesterone to estrogen ratios) have the highest renin-like activity [27]. Similarly, Husain's group has reported that AII receptors are most concentrated over atretic follicles in rats [28]. However, AII causes increased estrogen secretion from quartered rat ovaries [29], supporting this as a mechanism by which granulosa cells can escape the atresia process induced by theca androgens.

(b) Granulosa cell proliferation. Estrogen, in conjunction with FSH, has a crucial role within the follicle during this phase of growth [26]. The estrogens can bind to receptors in the granulosa cells which are then stimulated to proliferate. In studies on adrenal gland cells, AII has been observed to be involved in increased nucleic acid and protein synthesis, cell growth and cell proliferation [30, 31]. Thus, in the ovary, locally produced AII could participate in the regulation of stromal, thecal, granulosa and luteal cell growth and proliferation.

Late follicular development: The preovulatory phase

(a) Granulosa dominance. During this stage, the granulosa cells saturate the follicle with estrogen and it begins to rise in the peripheral circulation. Although the vessels that are destined to penetrate the basement membrane are still in formation, the levels of estrogen that reach the circulation are rising. Inside the follicle, estrogen results in the formation of a defined cumulus with secreted mucopolysaccharides and gap junctions between cells [32]. Estrogen-induced cyclic AMP levels rise and appear to arrest the maturing oocyte from completing its final maturational division [33]. The vascularization of the follicle reaches toward completion and, with the penetration of the basement membrane by capillaries, there is an increased exportation of follicular estrogen into the blood. At

this point, although immunoreactive renin and angiotensin are observable in preovulatory ovarian granulosa cells [10], follicular fluid levels of angiotensin and renin do not yet exceed levels found in the blood [9]. The follicular fluid contains mostly prorenin which must be activated before it can produce AII [34]. Recent *in vitro* studies have shown that in addition to the ability of plasminogen activators to activate prorenin, direct cleavage of renin substrate to AII could occur [35]. Furthermore, evidence from our laboratory indicates that follicular development is impaired by inhibitors of serine proteases [36]. Because of the known association between plasminogen activators and tissue remodeling and differentiation, it is possible that FSH induction of plasminogen activation may also be related to this stage of follicular development. Since plasminogen activator may be important in the activation of renin, it is also possible that this aspect of follicular development is, to a degree, under the control of angiotensin. In this regard, it is interesting to note that during the stage of granulosa cell dominance, the follicular fluid contains high levels of both plasminogen activator and plasminogen activator inhibitor [37, 38]. This circumstance would allow the follicular fluid to act as a reservoir holding the inactive components of the angiotensin-generating system, setting the stage for a massive increase in AII late in the preovulatory period [9].

The stage of granulosa dominance comes to a close with the completion of the vascularization of the follicle and the dramatic rise of circulating estrogen levels. There are indications that angiotensin induces the secretion of estrogen by the follicle [29, 39]. It is the dramatically increased secretion of estrogen into the blood which signals the appropriate synchronization of maturational events within the follicle and results in the surge of the ovulating hormone, LH [40].

(b) Granulosa failure/early luteinization. The surge of LH causes terminal follicle growth and oocyte expulsion from the follicle. This is accompanied by granulosa cell failure to maintain estrogen secretion, early luteinization of the granulosa cells, and final maturation division of the oocyte. We have evidence that human granulosa-luteal cells cultured in the presence of AII have an increased aromatase activity at 10^{-11} and 10^{-10} M, but that 10^{-9}–10^{-6} M AII causes diminishing increases in induced aromatase [39], suggesting that while increasing follicular AII initially drives the granulosa cell dominance, following the LH surge the further rise of AII contributes to granulosa cell failure. The fall of E_2 results in loss of gap junctions between cumulus cells and the completion of oocyte maturation: germinal vesicle breakdown. The abrupt rise of AII could be achieved, as mentioned above, by the activation of renin via the plasminogen activator pathway [35]. Levels of angiotensin exceeding 10^{-10} M are common in preovulatory follicular fluid; however, there have been contradicting data regarding the functional state of the granulosa cells of preovulatory follicles. Speth *et al.* [12] have reported that AII receptors exist in both immature and mature ovarian follicles of the rat. Also, granulosa cells are known to contain receptors to AII [11, 41]. Our group has demonstrated, by immunocytochemical localization, AII in human preovulatory granulosa cells [10]. However, studies by Daud *et al.* [28] did not show AII receptors on preovulatory follicles in gonadotrophin-treated immature rats. We have been able to diminish by approximately 60% human chorionic gonadotrophin (hCG)-induced germinal vesicle breakdown (GVBD)* and hCG-induced ovulation [42] in gonadotrophin-stimulated immature rats by the administration of saralasin, an angiotensin II receptor antagonist. Simultaneous administration of receptor-saturating quantities of AII restored normal rates of GVBD and ovulation. Additional data have been obtained by Catt's group who showed elegantly that endogenous AII receptors could play a role in the regulation of oocyte maturation [43]. The findings regarding ovulation have been questioned by Husain's group because of their receptor data and their inability to reproduce the results with saralasin [44]. In restudying the effects of saralasin on ovulation in gonadotrophin-stimulated prepubertal rats, we concluded that the irregularity of the results stems from differences in maturity and weight of the test animals and in the precision of responses for each of the biologically derived hormone preparations used [45]. We have analyzed carefully the composition of the different reagents used and found that in the case of hCG, different commercial preparations contain a variable and often major component of radioimmunoassable hCG that is not biologically active (β core fragment, βCF).† This raises serious experimental and clinical questions.

An *in vitro* model for investigating the direct ovarian response to AII has been used independently by two other groups. Peterson *et al.*, using an *in vitro* perfusion system, clearly showed a direct inhibitory effect of saralasin on the rat ovary even greater than the one observed with our *in vivo* model.‡ Furthermore, they were able to abolish the effect of saralasin by the simultaneous administration of 1.0 μM AII.§ Wallach's group, utilizing the *in vitro* perfused rabbit ovary, was able to induce ovulation with AII in the absence of gonadotrophin.‖ Furthermore, they demonstrated that saralasin

* Palumbo A, Pellicer A, DeCherney AH and Naftolin F, Angiotensin action in oocyte maturation in the rat. Abstract presented at the Society for Gynecologic Investigation meeting, 1988.

† Andrade-Gordon P, Apa R, Zreik T, Kardana A, Cole LA and Naftolin F, β Core fragment, the principal immunoreactive molecule in commercial hCG preparations is not biologically active. The 72nd Endocrine Society Meeting. Abstract 650, p. 187, 1990.

‡ Peterson CM, Zhu C, Makaida T and LeMaire WJ, The angiotensin II antagonist, saralasin, inhibits ovulation in the perfused rat ovary. Abstract 25 presented at the 22nd Annual Meeting of the Society for the Study of Reproduction, Columbia, MO, 1989.

§ Peterson CM, Zhu C, Mukaida T, Butler TA, Woessner JF and LeMaire WJ, The angiotensin II antagonist, saralasin, inhibits ovulation in the perfused rat ovary. Manuscript submitted for publication.

‖ Dharmarajan AM, personal communication, cited with permission.

blocked the AII-induced ovulation, suggesting the presence of AII receptors in the preovulatory follicles of the mature rabbit and indicating a possible direct role of AII in the process of ovulation. Moreover, the finding by Daud *et al.* [44] of an apparent absence of AII receptors on the preovulatory rat follicle at a time of massive increases in AII secretion does not negate direct involvement of AII in ovulation. While our understanding of the exact mechanism by which AII acts on the preovulatory follicle to stimulate the ovulatory process remains to be elucidated, the evidence strongly supports the concept that intraovarian events can be modulated by manipulation of the OVRAS.

In summary, it is evident that while the picture of the function of OVRAS remains incomplete, the evidence so far demonstrates the existence of an intrinsic OVRAS which is an important regulatory system for ovarian function. But there is an ever-changing group of intraovarian organelles and, depending upon the stage of the compartmental development of the ovary, there are different roles for angiotensin. The OVRAS is under gonadotrophin control. AII seems to play a role in follicle development, possibly through the modulation of follicular steroidogenesis. In the bulk of activated follicles this results in atresia. In dominant follicles the high preovulatory levels of AII in follicular fluid appear to be involved in the maturation of the oocyte and in ovulation, either directly or through other ovarian regulators, such as plasminogen activators. Clearly, exciting new discoveries related to OVRAS are still awaiting which will help to clarify the role that this system plays in ovarian function and development.

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