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GRANULOSA CELL-THECA CELL INTERACTIONS DURING FOLLICULAR DEVELOPMENT

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INTRODUCTION

In the structuring of a tissue, cells communicate to promote morphogenesis and cytodifferentiation. Cell-cell interaction can occur in a number of different ways: 1) through gap junctions formed between adjacent cells, 2) the production of cell adhesion molecules (CAM), 3) the formation of extracellular matrix (ECM) components or 4) by the secretion and transport of specific factors to neighboring cells (Fig.1). The type of cell-cell interaction which can occur in the formation of a functional unit is exemplified by the influence of mesenchymal cells on epithelial morphogenesis and differentiation (eg. uterus, prostate, mammary gland) (1).

We propose that the preovulatory follicle completes its development program as a result of similar interactions between thecal cells of mesenchymal origin and granulosa cells of epithelial origin. Since thecal cells and granulosa cells are not directly in contact they are unable to communicate through gap junctions and cell adhesion molecules. We have pursued the concept, therefore, that secreted factors such as steroids, peptides, proteins and components of extracellular matrix may be the essential local modulators of follicular development. This hypothesis is supported by the established role of androgens produced by the thecal cells in augmenting FSH-induced aromatase and cholesterol side-chain cleavage activities (2,3).

The role of proteins in local regulation was suggested by the production of an angiogenic factor by rat follicles (4) and its presence in human follicular fluid (5). Furthermore, thecal cells produced a component which promoted the proliferation of 3T3 and granulosa cells (6). Dižerega et al. found a protein in the venous drainage of the preovulatory ovary and in human follicular fluid which suppressed the follicular response to gonadotrophins and proposed that this inhibitory factor may be involved in atresia (7,8). In this paper we provide evidence that fibronectin is secreted by unstimulated immature granulosa cells and propose that this component of ECM may play a role in cell-cell communication and follicular development.

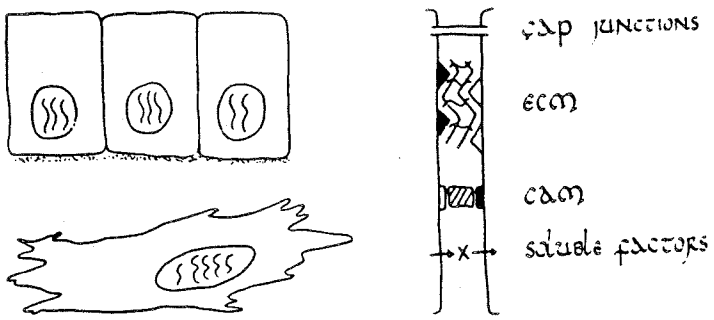

Fig.1: Mechanisms by which cells can communicate

RESULTS AND DISCUSSION

Our studies on rat granulosa cells suggest that "insulin-like" factors may be involved in modifying FSH-dependent cellular differentiation. FSH-stimulates aromatase activity, assessed by the release of tritiated water from [1β-^{3}H]testosterone, in granulosa cells isolated from DES-treated 25 day rats (9, Fig.2). Insulin, when added to a suboptimal concentration of FSH, produced a synergistic effect.

The concentration of insulin required was higher than that found in plasma, suggesting that if these effects are important physiologically then "insulin-like" factors may be produced locally within the follicle. This idea was further supported by the finding that conditioned medium obtained from cultures of rat thecal cells augmented FSH-induced aromatase activity. Thecal cell conditioned medium at a concentration of 10% produced a maximum augmentation which was comparable to that produced by insulin.

Another approach used to identify intraovarian factors was to examine, by electrophoresis, the [^{35}S]methionine radiolabelled proteins secreted by rat granulosa cells. A protein of molecular mass 220,000 daltons increased in intensity only in control cultures and became the major secreted protein after 72h, comprising 20% of the total radiolabelled proteins (10). This protein was identified as fibronectin by immunoprecipitation and measured by an ELISA assay. The

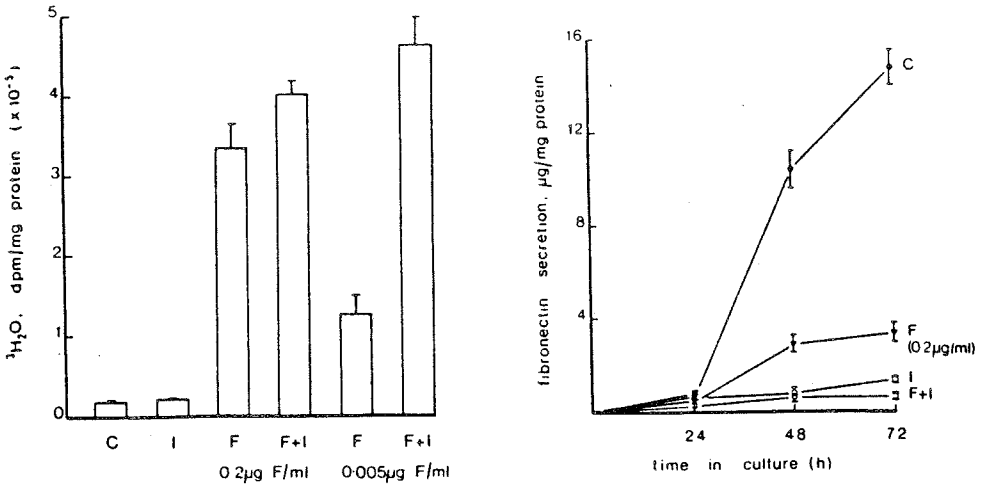

Fig. 2. Effects of NIH-FSH- 15 (F) and 5 ug insulin/ml (I) on A. aromatase activity and B. cumulative fibronectin secretion. After 48h in culture aromatase activity was assessed by incubating cells for 1h with 0.2uCi [1ß-^{3}H]testosterone (0.25uM) and measuring the amount of ^3H_2O released. Each value is a mean ± SE(n=3).

hormonal regulation of fibronectin secretion was investigated using the ELISA assay. As shown in fig.3, granulosa cells from 25 day old DES-treated rats cultured under control conditions secreted low levels of fibronectin during the first 24h of culture after which there was a rapid increase in secretion until 72h. In contrast, both FSH and insulin independently suppressed the increase in fibronectin secretion found in control cultures. Testosterone and estrogen alone did not influence fibronectin secretion and did not modulate the actions of FSH and insulin.

Fibronectin is a component of ECM and plays an important role in cell adhesion, migration and cell shape. These functions are due to its ability to bind to collagen, heparin and the cell surface (11). Fibronectin can also influence normal cytodifferentiation as illustrated by its inhibitory effect on myoblast fusion and glycosaminoglycan synthesis by chondrocytes (11). In addition, fibronectin can regulate adipocyte differentiation by inhibiting normal gene expression of lipogenic proteins (12). During the early stages of follicular development fibronectin may be important for the formation of the correct granulosa cell-theca cell structural arrangement and also influence the cytodifferentiation of either cell type. The inverse relationship between fibronectin secretion and the induction of those

granulosa cell functions essential for the development of the preovulatory follicle (eg. aromatase activity) indicates that fibronectin may provide a useful marker for the stage of cytodifferentiation and follicular maturation.

In summary, thecal cells and granulosa cells communicate by the production of steroids and proteins in order to promote the development of the preovulatory follicle and establish an environment in which the oocyte can develop normally.

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