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Rapid Paper

Mesenchymal-epithelial cell interactions in the ovary: estrogen-induced theca cell steroidogenesis

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Summary

The role of mesenchymal-epithelial cell interactions in the control of ovarian physiology was investigated Theca cells are the mesenchymal (i.e. stromal) like cells that surround the ovarian follicle and produce androgen in response to the gonadotropin luteinizing hormone (LH). Granulosa cells are the epithelial-like cells that form the follicle, support the developing oocyte, and utilize androgens produced by theca cells as a substrate for the production of estrogen Observations presented indicate that estrogen produced by granulosa cells dramatically stimulates androgen production by theca cells Estrogen was found to have greater stimulatory effect on theca cell androgen production than gonadotropin, and a combination of estrogen and gonadotropin results in a greater than additive response of the two hormones. Regulation of androgen production by estrogen provides a local feedback loop in the follicle that will significantly influence ovarian steroidogenesis. This steroid-mediated theca-granulosa cell interaction provides evidence for the importance of mesenchymal (i.e. stromal)-epithelial cell interactions in adult tissues and implies that epithelial cells can produce paracrine factors that modulate mesenchymal cell function and differentiation The theca cell-granulosa cell interaction identified is postulated to be a critical mesenchymal-epithelial cell interaction for the control of ovarian physiology and the endocrine status of the female.

Introduction

One of the most common cell-cell interactions that has evolved in mammals is between mesenchymal (i.e. stromal) cells and epithelial cells. Mesenchymal cells are known to influence the differentiation of epithelial cells during embryonic development (Grobstein, 1967; Cunha et al., 1983) and most functioning epithelial cell types in adult tissue are in contact with mesenchymal (i.e. stromal) cells. Therefore, mesenchymal-epithelial cell interactions appear to be important for both embryonic development and adult tissue function. This cellular interaction is more commonly referred to as a stromal-epithelial cell interaction in adult tissue; however, several tissues such as the ovarian follicle are distinct and appear to undergo an embryonic type development in adult tissue. The ovary provides a useful tissue to investigate cell-cell interactions because individual cell types with known differentiated functions can

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be isolated. The developing follicle within the ovary is made up of several cell types including theca cells, granulosa cells and the oocyte. The theca cells are mesenchymal (i.e. stromal)-like cells that surround the follicle and are in contact with the outer layer of epithelial-like granulosa cells. Theca cells are derived from the stromal-interstitial cell population and are induced to differentiate at the onset of follicle development.

Both theca cells and granulosa cells are sites of action for the pituitary gonadotropins and synthesize ovarian steroid hormones. In species such as the human, bovine and rat, theca cells produce androgens and progestins while granulosa cells produce estrogens and progestins. The initial steroid-mediated theca cell-granulosa cell interaction identified involved the 'two cell hypothesis' (Flack, 1959; Short, 1963; Ryan et al., 1968). Ovarian theca cells produce androgens that are subsequently utilized by granulosa cells as a substrate for the production of estrogen by the P450 enzyme aromatase (Dorrington et al., 1975; Armstrong and Papkoff, 1976; Wang et al., 1981). The estrogen produced by granulosa cells, therefore, is dependent on the supply of androgen by theca cells. Estrogen can feedback on the pituitary/hypothalamus to regulate gonadotropin production and estrogen can also act as an autocrine factor to regulate the actions of follicle stimulating hormone (FSH) on granulosa cells (Richards et al., 1976; Hsueh et al., 1984). Estrogen production by granulosa cells has also been shown to influence progestin production by theca cells (Fortune and Hansel, 1979; Fortune, 1986) and progestins produced by granulosa cells appear to act as substrate for theca cell production of androgens (Fortune, 1986). Although other factors have been shown to influence theca cell steroidogenesis (Hernandez et al., 1986; Tonetta et al., 1986), LH has been postulated to be the primary regulatory agent involved in the control of theca cell function (Baird et al., 1976; McNatty et al., 1984; Richards et al., 1986).

Materials and methods

Cell culture preparation and culture conditions. Theca cells were isolated from bovine ovaries and cultured as previously described (Skinner et al., 1987; Skinner and Coffey, 1988). Bovine ovaries were collected from young non-pregnant cycling heifers slaughtered at an abattoir and delivered fresh on ice by Research Supply, Nashville, TN, U.S.A. Follicles ranging in size from approximately 2 mm to 15 mm in diameter were dissected from the ovaries and classified as healthy or atretic as previously described (McNatty et al., 1984; Skinner et al., 1987; Skinner and Coffey, 1988). Follicular fluid was aspirated from follicles and follicles were bisected. Granulosa cells were removed from the follicle wall by scraping with a fine plastic loop and flushing back and forth in Ham's F-12 medium. Theca interna layers were then microdissected away from the theca externa and cleaned of any adhering granulosa cells. Theca interna layers were minced into small pieces and digested for 1 h at 37 °C in Ham's F-12 containing 1 mg/ml collagenase, 1 mg/ml hyaluronidase, 1 mg/ml pronase and 0.01 mg/ml DNAse. Dispersion of cells was facilitated by agitating the solution back and forth through a Pasteur pipette at 15–20 min intervals during the digestion. Dispersed cells were centrifuged for 4 min at $40 \times g$, resuspended in medium and plated in 1 ml Ham's F-12 in 24-well Linbro plates. Cells were cultured at 37 °C in a 5% CO_2 atmosphere with treatments and durations which are outlined in the Results section. Cell preparations obtained by this procedure have been characterized cytochemically to contain less than 5% contamination with endothelial and/or granulosa cells.

Steroid and DNA assays. At the end of each culture period, medium was removed from cells, centrifuged to remove cellular debris and frozen for storage until assayed for steroid content. Fresh medium was then added to cells for further culture or cells were terminated and DNA content/culture well was determined fluorometrically with ethidium bromide as previously described (Skinner et al., 1988).

The steroids quantitated in this study were androstenedione, progesterone and testosterone. Progesterone was quantitated by radioimmunoassay as described previously (Skinner and Osteen, 1988) and androstenedione was quantitated with similar procedure using an ANG-22 antibody (Endocrine Sciences, Tarzena, CA, U.S.A.). Testosterone was quantitated utilizing the Coat-A-Count

Total Testosterone radioimmunoassay kit purchased from Diagnostic Products Corporation, Los Angeles, CA, U.S.A. All concentrations of steroids reported in this paper have been normalized to DNA content present in the respective culture wells. Estradiol did not cross-react with the antibodies used in the radioimmunoassays.

Statistical analysis. A SAS analysis of variance procedure for a randomized block design, using experiment replicate as the blocking factor was used to analyze all data and a protected Fisher's LSD procedure was used to make pairwise comparisons. Progesterone data were transformed by taking the square root of progesterone values because of heterogeneity of variance. Each study contained 3–4 experiments with 2–3 replicates per experiment.

Results and discussion

Investigation of the regulation of theca cell function revealed a novel steroid-mediated mesenchymal-epithelial cell interaction between theca and granulosa cells. Purified populations of bovine theca cells were placed in serum-free cell culture and the ability of estrogen to influence cellular steroidogenesis examined. Human chorionic gonadotropin (hCG), which mimics the actions of LH (Magoffin and Erickson, 1982), stimulates androgen production by theca cells approximately 2-fold (Fig. 1). Interestingly, estradiol alone was found to have a more dramatic effect on theca cell androgen production with a 5-fold stimulation (Fig. 1). Estradiol stimulated the production of both androstenedione and testosterone; however, the levels of testosterone produced by theca cells was approximately an order of magnitude less than androstenedione. A combination of estradiol and hCG resulted in a greater than additive response with a 10-fold stimulation of androstenedione production by theca cells (Fig. 1). In contrast to the ability of estradiol to stimulate androgen production, estradiol was found to suppress basal levels slightly and decrease the ability of gonadotropin to stimulate progesterone production (Fig. 2). The minimal concentration of estradiol required to stimulate androstenedione production by cultured theca cells was 10^{-9} M while the optimal concentrations were between

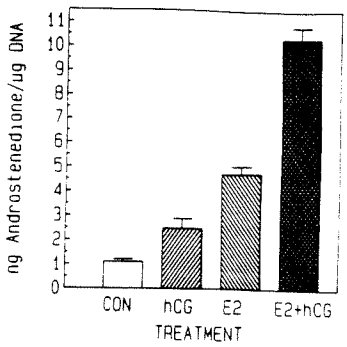

Fig. 1. Hormonal regulation of androgen production by bovine ovarian theca cells. Theca cells were cultured in the absence (CON) or presence of human chorionic gonadotropin, 1 µg/ml (hCG), or estradiol, 10^{-6} M (E_2). Conditioned medium was collected after 3 days of culture and the quantity of androstenedione produced determined with a radioimmunoassay. All data are normalized per µg theca cell DNA. Data are presented as the mean ± SEM from four different experiments done in triplicate. All treatments were statistically different from each other ($P < 0.05$).

10^{-7} and 10^{-6} M (Fig. 3). The concentration of estrogens present in the developing ovarian follicle ranged from 10^{-8} M in small follicles to 5×10^{-7} M in large follicles (Henderson et al., 1982; Grimes and Ireland, 1986; Wise, 1987; Zimmerman et al., 1989). Therefore, estrogen concentrations in

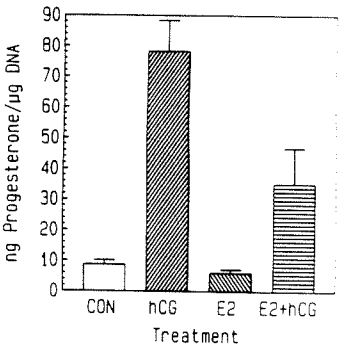

Fig. 2. Hormonal regulation of progesterone production by bovine ovarian theca cells. Theca cells were cultured and treated as outlined in the legend to Fig. 1. Progesterone production by theca cells in serum-free culture is non-responsive to gonadotropin on day 3 of culture (Fortune, 1986). Therefore, conditioned medium was collected between days 3 and 6 of culture for analysis of progesterone levels with a specific radioimmunoassay and all data normalized per µg theca cell DNA. Data are presented as the mean ± SEM from four different experiments done in triplicate. All treatments were statistically different from each other ($P < 0.05$).

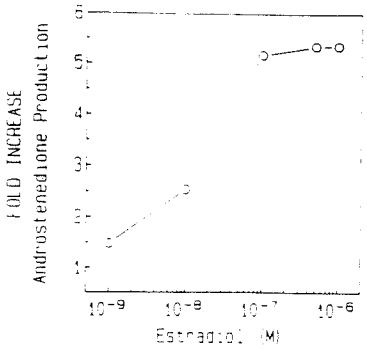

Fig. 3. Bovine theca cells were cultured in the presence of various concentrations of estradiol. Conditioned medium was collected on day 3 of culture and the amount of androstenedione present determined and normalized per μg theca cell DNA. Data are expressed as fold increase over the level of androstenedione produced by control non-treated cells. A representative experiment is presented from three different experiments.

ovarian follicles are sufficient to influence theca cell steroidogenesis. These observations indicate that estrogen produced by granulosa cells can act on theca cells to stimulate the production of androgens that can subsequently be used by granulosa cells for the production of estrogen (Fig. 4). This local feedback loop is postulated to play an important role in the control of ovarian function. The ability of estrogen to have a more dramatic effect on androgen production than hCG/LH, and a greater than additive response with the combination of estrogen and hCG/LH, provides evidence that estrogen may have an essential role in the regulation of theca cell steroidogenesis. Estrogen appears to induce theca cell differentiation to an androgen producing cell that

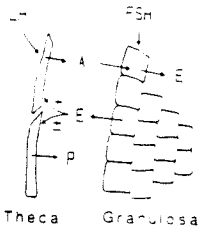

Fig. 4. Schematic of the proposed steroid-mediated interactions between ovarian theca cells and granulosa cells. The steroids involved include androgens (A), estrogens (E) and progestins (P) with both positive (+) and negative (−) effects on steroidogenesis. The gonadotropins involved include luteinizing hormone (LH) and follicle stimulating hormone (FSH).

will be important during development of preantral follicles to large antral follicles. As previously reported, high concentrations of estrogen can inhibit theca cell progesterone production while low concentrations stimulate progesterone production (Fortune and Hansel, 1979). Therefore, the role estrogen may have in regulating theca cell steroidogenesis during the postovulatory stage of follicle development when progestin production is predominant is unclear. The effects of follicle development on the actions of estrogen remain to be investigated.

The endocrine control of female reproduction is dependent on the actions of gonadotropins on ovarian follicle cells. Local regulation of ovarian cell function through cell-cell interactions, however, will likely have an equally important role in the control and maintenance of female reproduction. These data provide evidence that the combined actions of estrogen and LH may be necessary to induce and regulate ovarian theca cell steroidogenesis. The observations presented in the current report support the importance of mesenchymal-epithelial cell interactions in the control of tissue growth and differentiation. Although the ability of mesenchymal cells to produce inducer substances which influence the adjacent epithelial cells has been demonstrated (Grobstein, 1967; Cunha et al., 1983; Skinner et al., 1988), the ability of the epithelial cells to influence the differentiation of the adjacent mesenchymal cells is not fully appreciated. Results presented demonstrate that epithelial cells, granulosa cells, can produce a paracrine factor, estrogen, that influences the differentiation of the adjacent mesenchymal cells, theca cells. Information available implies that the mesenchymal cells and epithelial cells will form a functional unit to regulate tissue function. This cell-cell interaction is speculated to be essential for the cellular physiology of a large number of different tissues and organs. The current report supports the postulate that mesenchymal (i.e. stromal)-epithelial cell interactions are important for both embryonic and adult tissue physiology.

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