Endocrine (1995) 3, 107–112

Preantral follicles stimulate luteinizing hormone independent differentiation of ovarian theca-interstitial cells by an intrafollicular paracrine mechanism*

Denis A. Magoffin & Paul C. Magarelli[1]

Department of Obstetrics and Gynecology, Cedars-Sinai Research Institute, Cedars-Sinai Medical Center/UCLA School of Medicine, Los Angeles, California 90048, USA

Ovarian thecal cells are thought to differentiate from fibroblast-like precursor cells in the stroma adjacent to developing follicles. Since the precursor cells do not contain LH receptors, a regulator other than LH must initiate thecal differentiation. These studies were designed to test the hypothesis that preantral follicles secrete substances that can stimulate thecal differentiation. Preantral follicles devoid of theca were obtained by limited enzymatic dispersal of 26-day old rat ovaries. Follicles were cultured (5 follicles/well) in 96-well plates containing serum-free medium to generate follicle-conditioned medium (FCM). Isolated theca-interstitial cells (TIC) were cultured (2 days) in 50% FCM, to bioassay for androgen-stimulating activity. Androsterone production was measured by RIA. FCM from follicles of increasing size with 1, 2, 3, 4 or 5 layers of granulosa cells (GC) stimulated increasing amounts of androsterone suggesting that secretion of androgen-stimulating activity is developmentally regulated in preantral follicles. The androgen-stimulating activity of 7.5-fold concentrated FCM was markedly increased above control levels or the levels stimulated by insulin-like growth factor-I (100 ng/ml), transforming growth factor-α (100 ng/ml), transforming growth-factor-β1 (10 ng/ml), inhibin A (300 ng/ml), activin A (100 ng/ml), or Müllerian inhibiting substance (MIS; 300 ng/ml) suggesting that the bioactive substances were distinct from these intrafollicular growth factors. rFSH stimulated a >10-fold increase in androgen-stimulating activity demonstrating that the bioactivity is hormonally regulated. The bioactivity was sensitive to trypsin digestion but was not inhibited by indomethacin (10 μM) suggesting that it is peptide not prostaglandin in nature. Gel filtration chromatography indicated that the M_r of the bioactive peptides in FCM ranged from 19 500 to 23 600. Taken together our results demonstrate that preantral follicles secrete thecal differentiating factors (TDFs) that are developmentally and hormonally regulated by FSH. The properties of the TDFs are markedly different from known intrafollicular growth factors and may represent a new paracrine regulator in the ovary that can stimulate LH-independent thecal differentiation.

Keywords theca-interstitial cells; follicle development; androgen production

Correspondence: Denis A. Magoffin
[1]Present address: Department of Obstetrics, Gynecology and Reproductive Biology, B316 Clinical Center, Michigan State University, East Lansing, MI 48824-1315
*This research was supported by NICHD Grant HD-28154.
Received 13 September 1994; accepted 3 October 1994.

Introduction

Differentiation of the ovarian theca cells involves the sequential expression of a variety of characteristic genes, some of which are known and others remain to be elucidated. The theca cells are thought to originate from morphologically indeterminate, fibroblast-like precursor cells located in the ovarian stroma adjacent to primordial follicles (Erickson *et al.*, 1985). The undifferentiated pre-theca cells have not yet expressed LH receptors and are therefore not responsive to LH (Magoffin *et al.*, 1994). There is, however, evidence that gene expression is activated in pre-theca cells when primordial follicles are recruited into the pool of growing follicles (Hirshfield, 1991). Genes for LH receptors and steroidogenic enzymes are expressed prior to morphologic differentiation and before the differentiating theca become responsive to LH (Magoffin *et al.*, 1994). The first morphologically identifiable theca cells become associated with developing follicles when the follicle acquires 2–3 layers of granulosa cells (Ingram, 1959) and is coincident with the appearance of LH-responsiveness and low levels of steroidogenic enzyme activity in the newly differentiated theca (Magoffin *et al.*, 1994).

The observation that low levels of LH receptor and steroidogenic enzyme gene expression occurs before the differentiating theca can respond to LH suggests the hypothesis that substances other than LH initiate the genetic program of thecal differentiation. Since differentiated theca are only associated with growing follicles, it seems reasonable to propose that the growing follicle secretes one or more substances that regulate thecal differentiation independent of LH.

The purpose of the present studies was to test the hypothesis that preantral follicles secrete one or more substances that can stimulate thecal differentiation. To accomplish this goal we used theca-interstitial cells (TIC) isolated from immature hypophysectomized rats by Percoll gradient centrifugation (Magoffin & Erickson, 1988). The freshly isolated TIC are initially responsive to LH with increased cAMP production but LH does not stimulate steroidogenesis (Magoffin & Erickson, 1988; Magoffin, 1989). Even when substrates such as 25-hydroxycholesterol are provided to the freshly isolated TIC steroidogenesis is not increased demonstrating that the steroidogenic enzymes are not present in sufficient amounts to actively synthesize steroids (Magoffin *et al.*, 1990). Approximately 20 h of continuous stimulation with LH are required before mRNA for cholesterol side chain cleavage cytochrome

P450 ($P450_{SCC}$), 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17α-hydroxylase/C_{17-20} lyase cytochrome P450 ($P450_{17\alpha}$) mRNAs are expressed and translated into functional proteins (Magoffin, 1989; Magoffin & Weitsman, 1993a,b,c). In this model significant increases in androgen production occur only when steroidogenic enzyme gene expression is induced. In these studies we have used the isolated TIC as a cell culture bioassay for substances secreted by preantral follicles that can stimulate thecal differentiation.

Results

To determine if preantral follicles secrete substances that stimulate androgen production by ovarian theca-interstitial cells (TIC), isolated TIC were cultured in the presence of preantral follicle-conditioned medium (FCM). Follicles of increasing size from one through five layers of granulosa cells (GC) were cultured for 2 days in serum-free medium to generate FCM. TIC were then cultured in 50% FCM for 2 days to bioassay for androgen-stimulating activity. As shown in Figure 1, control TIC cultured in medium incubated without follicles for 2 days produced approximately 3 ng/ml of androsterone. FCM from follicles with two or more layers of GC stimulated androsterone production over control levels. These results demonstrate that preantral follicles secrete substances that stimulate thecal androgen production and that the amount of stimulating activity increases with follicle development.

We next compared the androgen-stimulating capacity of FCM with several substances produced by ovarian follicles that have been shown to regulate TIC androgen production. As shown in Figure 2, LH (0.3 ng/ml) and prostaglandin E_2 (PgE_2; 300 ng/ml) stimulated 30-fold increases in androsterone production ($P<0.001$). Saturating concentrations of insulin-like growth factor-I (IGF-I; 100 ng/ml), transforming growth factor-α (TGFα; 100 ng/ml), transforming growth factor-β1 (TGF-β1; 10 ng/ml), inhibin A (300 ng/ml), activin A (100 ng/ml), or Müllerian inhibiting substance (MIS; 300 ng/ml) did not alter basal androsterone production. In contrast, FCM from preantral follicles concentrated 7.5-fold caused a marked stimulation (18-fold) of TIC androsterone production over basal levels ($P<0.001$). These results strongly suggest that the androgen-stimulating substance in FCM is distinct from IGF-I, TGFα, TGF-β1, inhibin A, activin A and MIS but does not rule out the possibility that it is PgE_2 or cAMP.

The only substances previously shown to stimulate large increases in androgen production by ovarian TIC are LH/hCG, PgE_2, and substances that increase intracellular cAMP such as cAMP analogs, forskolin and cholera toxin. Ovarian follicles are not known to secrete gonadotropins, forskolin or cholera toxin, but they can produce prostaglandins and cAMP in response to FSH. To determine if prostaglandins were a component of the androgen-stimulating activity in FCM preantral follicles were cultured in the presence and absence of FSH and indomethacin, an inhibitor of prostaglandin synthesis. As shown in Figure 3, FCM from FSH-treated follicles stimulated a dramatic increase in adrosterone production by TIC. Incubation of the preantral follicles with indomethacin (10 μM) did not inhibit the androsterone-stimulating capacity of FCM from control or FSH-treated follicles. These results strongly suggest that products of arachidonic acid metabolism such as prostaglandins are not an important androgen-stimulating component of the FCM.

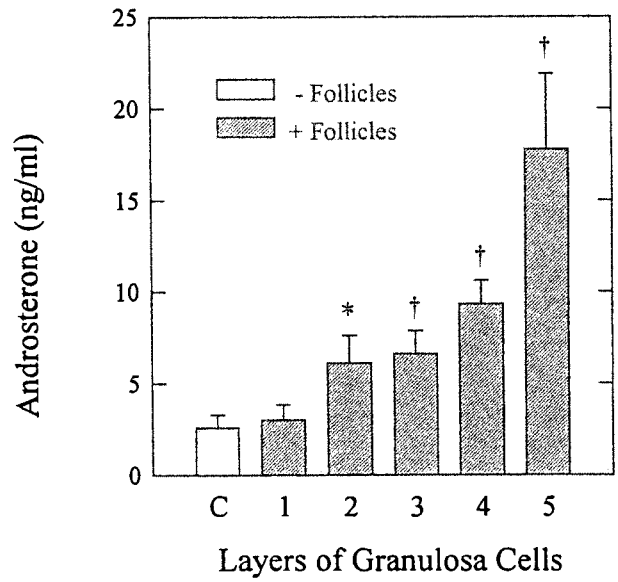

Figure 1 Production of androgen-stimulating activity by preantral follicles. Preantral follicles (5 follicles/well) with 1–5 layers of GC were cultured (2 days) in serum-free medium. Conditioned medium was diluted with an equal volume (100 μl) of fresh medium and used to culture TIC (5×10^4 viable cells/well) for 2 days. Androsterone in the medium was measured by RIA. The data are the mean ± SEM of three separate experiments with 2–3 replicates/experiment. *$P<0.03$ *vs* control; †, $P<0.01$ *vs* control

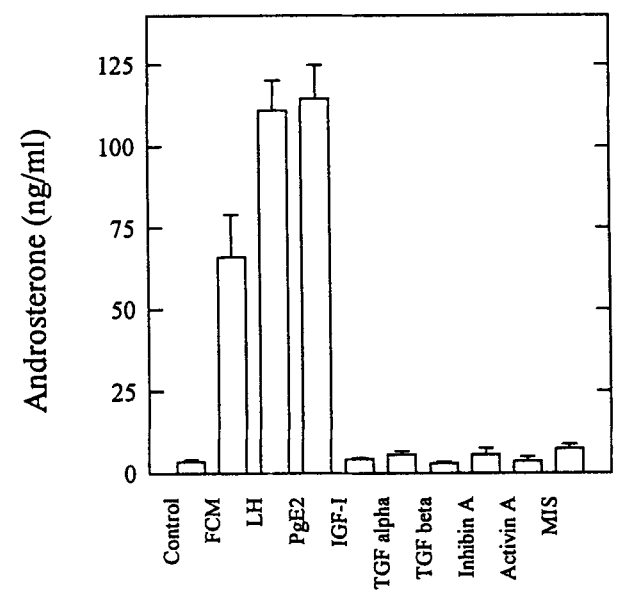

Figure 2 Comparison of the androgen-stimulating activity of FCM with intrafollicular regulatory molecules. TIC (5×10^4 viable cells/well) were cultured (2 days) with medium alone (Control), FCM from follicles with 3–5 layers of GC (100 μl, concentrated 7.5-fold), LH (0.3 ng/ml), PgE_2 (300 ng/ml), IGF-I (100 ng/ml), TGF-α (100 ng/ml), TGF-β1 (10 ng/ml), inhibin A (300 ng/ml), activin A (100 ng/ml) or MIS (300 ng/ml). Androsterone in the medium was measured by RIA. The data are the mean ± SEM of three experiments with 4 replicates/experiment

To test the hypothesis that the active substance in FCM was a protein, FCM from FSH-stimulated follicles was digested (2 h at 37°C) with trypsin (1 mg/ml). After the incubation trypsin activity was neutralized with soybean trypsin inhibitor (10 mg/ml) to prevent digestion of TIC in the subsequent bioassay. The digested FCM (100 μl) was tested for androgen-stimulating activity in the TIC bioassay (Figure 4). Control medium incubated with rFSH but no follicles had no effect on TIC androgen production demonstrating that the androgen-stimulating bioactivity was produced by the follicles and was not due to LH or other stimulatory activity in the FSH preparation. When follicles were treated with rFSH (0.1 IU/ml) there was a significant increase in bioactivity ($P<0.001$). Digestion of the FSH-treated FCM with trypsin eliminated the androgen-stimulating capacity of the FCM (Figure 4). Incubation of TIC with FCM containing soybean trypsin inhibitor alone did not diminish androsterone production (data not shown). To ensure that the trypsin activity was neutralized by the trypsin inhibitor, FCM was incubated with a mixture of trypsin plus inhibitor then bioassayed for androgen-stimulating activity. There was no alteration in androsterone production when the trypsin was neutralized prior to incubation. Thus, the androgen-stimulating activity in FCM appears to be a trypsin-sensitive peptide.

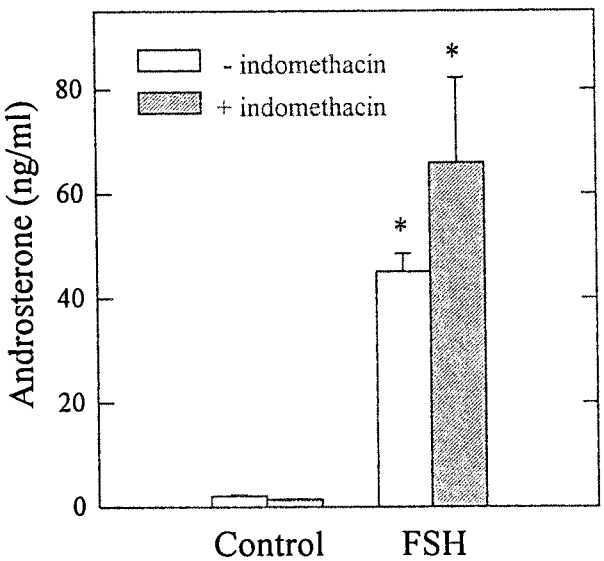

Figure 3 Effect of FSH and indomethacin on FCM stimulation of TIC androsterone production. Follicles with 1–5 layers of GC were cultured (2 days) with and without indomethacin (10 μM) or FSH (1 IU/ml). FCM was bioassayed for androsterone-stimulating activity. Data are the mean ± SEM of two experiments with 3 replicates/experiment. *, $P<0.01$ *vs* control

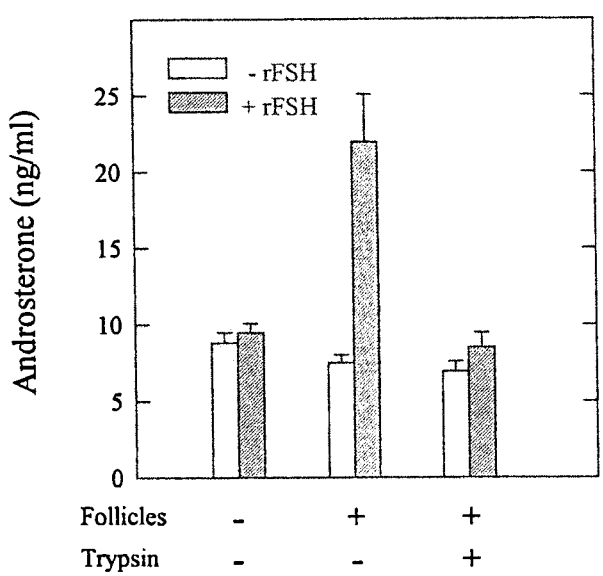

Figure 4 Effect of rFSH stimulation and trypsin digestion on androgen-stimulating activity of FCM. FCM from follicles (3–5 layers of GC) cultured with and without rFSH (0.1 IU/ml) was incubated with and without trypsin (1 mg/ml) for 2 h (37°C). After incubation soybean trypsin inhibitor (10 mg/ml) was added to neutralize the proteolytic activity. TIC (5×10^4 viable cells/well) were cultured in the treated FCM for 2 days. Androsterone in the medium was measured by RIA. The data are the mean ± SEM of two experiments with 3 replicates/experiment

To estimate the molecular weight (M_r) of the bioactive peptide(s) in FCM, we performed gel filtration chromatography of sixfold concentrated FCM from rFSH-stimulated follicle cultures. Bioassay of the fractions for androgen-stimulating activity revealed three fractions that exhibited substantial bioactivity (Figure 5) ranging from 19 500 to 23 600 M_r (Figure 6). These results suggest that preantral follicles secrete one or more peptides with androgen-stimulating activity. The M_r of the bioactive substances does not support the concept that cAMP, a prostaglandin, or a steroid hormone is the androgen-stimulating component of FCM but does not rule out the possibility of a family of cAMP- or prostaglandin-binding proteins.

Discussion

The results of our studies demonstrate that developing preantral follicles secrete paracrine substances with androgen-stimulating activity. Previous results with the

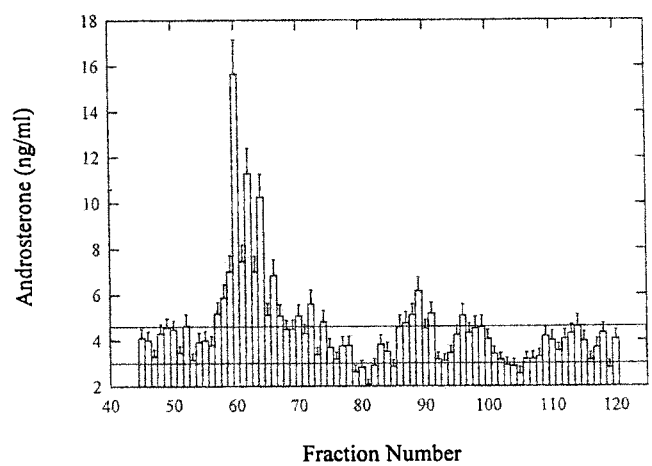

Figure 5 Gel filtration chromatography of FCM. FCM (600 μl) was concentrated sixfold then filtered through a sterile Biogel P-100 column equilibrated with Medium-199 containing 25 mM HEPES The fractions (45 μl) were added to TIC cultures for 2 days. Androsterone in the medium was measured by RIA. The horizontal lines represent the range of basal androsterone production. **a**, **b** and **c** are the three fractions for which M_r were calculated in Figure 6. The data are from a representative gel filtration experiment that has been replicated twice

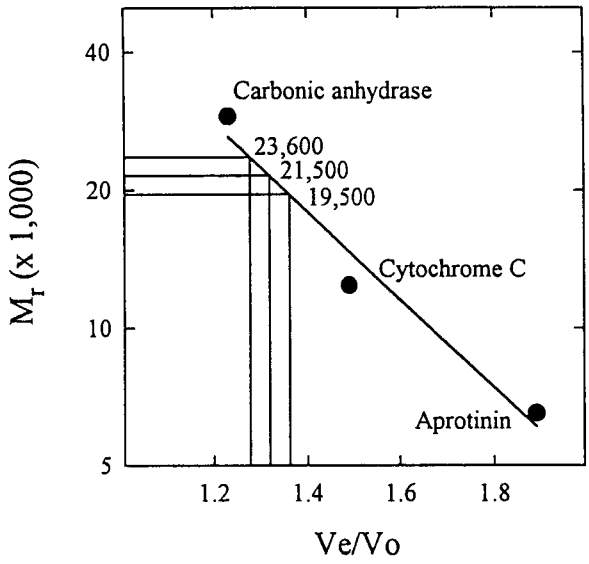

Figure 6 Determination of the M_r of the column fractions with androgen-stimulating bioactivity. The line is a linear regression of M_r *vs* Ve/Vo. The M_r of the bioactive fractions was calculated using the equation of the regression line and the measured Ve/Vo for each of the bioactive fractions (**a**, **b** and **c** as shown in Figure 5)

isolated TIC model have demonstrated that increases in androgen production of the magnitude observed with FCM occur only when expression of the steroidogenic enzymes in TIC is stimulated, (Magoffin & Erickson, 1988; Magoffin, 1989; Magoffin & Weitsman, 1993a, b, c). In the absence of increased steroidogenic enzyme expression the isolated TIC do not contain sufficient amounts of the enzymes to produce more than basal amounts of androgens. Consequently, it seems likely that the molecules secreted by preantral follicles stimulate the expression of steroidogenic enzyme genes in TIC and can therefore regulate TIC differentiation. Although our experiments do not directly examine the effects of the preantral follicle-secreted paracrine factors on TIC gene expression, it is tempting to speculate that these thecal differentiating factors (TDFs) may be involved in initiating the genetic program in the stromal precursor cells that leads to expression of the LH receptor and the characteristic steroidogenic enzymes present in the theca cells. Studies are currently in progress to determine the effects of the TDFs on LH receptor gene expression in TIC.

Our data clearly show that the follicle is the source of TDF, but the relative roles of the oocyte and GC are unclear. The observations that TDF activity is stimulated by rFSH and that increasing amounts of activity are produced as the number of GC in the follicles increases suggest that the GC may be the site of TDF production.

We have presented evidence that preantral follicle conditioned medium contains one or more peptides capable of stimulating large increases in thecal androgen production independent of LH or hCG. PgE_2 was the only previously known follicular substance capable of independently stimulating maximal androgen biosynthesis (Magoffin & Erickson, 1994). The bioactivity secreted by developing preantral follicles is destroyed by trypsin digestion and is unaffected by indomethacin suggesting that TDF is proteinacious in nature and not a prostaglandin. Our analysis suggests that there is one or more peptides produced with TDF activity in the 19k–24k M_r range. The selectivity of the gel filtration column used was not sufficiently high to unequivocally conclude that there was more than one peptide secreted. It is also possible that there are several isoforms of a single TDF protein. The M_r of TDF is in the same general range as intraovarian growth factors but distinct from a previously reported steroidogenesis-inducing protein (SIP) with a M_r of 60 k isolated from human follicular fluid (Khan *et al.*, 1990). Our data also appear to rule out a mechanism involving production of a steroid precursor for androgen production as has been previously reported (Lischinsky & Armstrong, 1982).

Although growth factors such as IGF-I (Magoffin & Weitsman, 1993a, b, c), TGF-β and inhibin (Magoffin *et al.*, 1994) have been shown to stimulate steroidogenic enzyme mRNA in TIC, none of the growth factors tested to date have stimulated high levels of androgen production. It is also unlikely that TDF is an IGF binding protein (IGFBP) or a mixture of IGF-I and one or more IGFBP. *In situ* hybridization studies in the rat have shown that none of the IGFBPs (IGFBP-1 through IGFBP-6) are expressed in the GC of healthy follicles at any stage of development including the preantral stages (Nakatani *et al.*, 1991; Erickson *et al.*, 1992a,b; Erickson *et al.*, 1993; Erickson *et al.*, 1994b,c). IGFBP-4 and -5 mRNAs are expressed in the GC of atretic follicles (Nakatani *et al.*, 1991; Erickson *et al.*, 1992a,b; Erickson *et al.*, 1994c). These two binding proteins are secreted by GC obtained from hypophysectomized rats *in vitro* (Adashi *et al.*, 1993; Liu *et al.*, 1993; Erickson *et al.*, 1994a), conditions in which the GC express the characteristics of atretic follicles (Sadrkhanloo *et al.*, 1987). In contrast, IGF-I mRNA is expressed only in the GC of healthy follicles in the rat (Oliver *et al.*, 1989) suggesting that GC can secrete IGF-I or IGFBPs depending on the health of the follicle but not both. The preantral follicles used in our studies were obtained from intact rats and were healthy when placed into culture, therefore they would be expected to secrete IGF-I but not IGFBPs. It is possible that the follicles might have become atretic during the culture period, in which case they might have stopped producing IGF-I and started secreting IGFBP-4 and -5.

Although the potential exists for the presence of IGF-I and IGFBPs in FCM, the data in the literature do not support the concept that either molecule or a combination of the two could be TDF. First, our data demonstrate that the bioactive components have a M_r in the range of 19–24 k. Both IGFBP-4 (M_r = 25 681) and IGFBP-5 (M_r = 28 428) are larger than this range. Furthermore, the IGFBPs have a high affinity for IGF-I under the conditions used for the gel filtration experiments and the IGF-I/IGFBP complex would not dissociate. Thus the M_r for the complexes would be on the order of 34 500–37 300, values that significantly exceed the observed M_r for TDFs. As we have shown in our studies IGF-I alone cannot mimic the stimulatory effects of FCM on TIC androgen production. In the ovary the effects of IGFBPs have been uniformly inhibitory with respect to gonadotropin-stimulated steroidogenesis and have not been shown to

stimulate steroidogenesis alone (Erickson *et al.*, 1994c). Thus it appears that TDF may be a novel molecule or combination of molecules. Additional studies are in progress to establish the identity of the TDFs.

We have shown that FSH markedly increases the androgen-stimulating capacity of preantral follicle conditioned medium. One striking implication of this finding is that thecal androgen production may be under the control of FSH. The two-cell, two gonadotropin concept for follicular estrogen biosynthesis states that thecal androgen production is under the regulation of LH and that aromatization of the androgen to estrogen is under the control of FSH. Our data provide a mechanism in the intact follicle whereby FSH might be able to stimulate not only aromatase activity, but also androgen substrate production through an indirect mechanism. Such a mechanism is attractive because the GC through secretion of paracrine mediators could communicate a need for androgen substrate to the theca interna simultaneously with the expression of aromatase activity and may play an important role in the selection of the dominant follicle. It will be important to determine if this mechanism functions in developing follicles.

Materials and methods

Hormones and reagents

Ovine LH (AFP-5551B) and recombinant human FSH (rFSH; R1) were a gift of the National Hormone and Pituitary Program. Human TGF-β1 and TGFα were from Collaborative Research (Bedford, MA). Recombinant human MIS was provided by Dr. David T. MacLaughlin, Massachusetts General Hospital (Boston, MA). Recombinant human activin-A (15365-23 (R)) was obtained from Genentech (South San Francisco, CA). Recombinant human inhibin-A (rh INH-R-90/1; 51 000 U/μg) was provided by Dr. R. G. Forage, Biotech Australia (E. Roseville, Australia). Recombinant human IGF-I was obtained from R&D Systems (Minneapolis, MN). Prostaglandin E_2 and soybean trypsin inhibitor were obtained from Sigma Chemical Co. (St Louis, MO). Trypsin and cell culture reagents were obtained from Gibco (Grand Island, NY).

Follicle culture

Preantral follicles were obtained by a modification of a previously described method (Roy & Greenwald, 1985). Briefly, the ovaries were dissected from the ovaries of 26 day old Sprague-Dawley rats euthanized by cervical dislocation after CO_2 asphyxiation as approved by the Institutional Animal Care and Use Committee. The ovaries, cut into four pieces, were dissociated with collagenase/DNase solution (Magoffin & Erickson, 1982) by incubating at 37°C for 40 min and flushing through a Pasteur pipet every 20 min. The dispersate was washed with Medium 199 containing 25 mM HEPES and 1 mg/ml BSA then filtered through a 500 μM mesh to remove large ovarian fragments. Preantral follicles were collected under a dissecting microscope using calibrated micropipets. This method, in agreement with previously published results (Roy & Greenwald, 1985), yielded intact follicles with no theca interna attached. Five follicles/well were cultured in 96-well tissue culture plates containing 200 μl McCoy's 5a medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin sulfate and 2 mM L-glutamine for 2 days at 37°C in a 95% air–5% CO_2 atmosphere. In experiments where follicles of multiple sizes were cultured in each well, the different sized follicles were randomly assigned to treatment groups. The follicle-conditioned medium (FCM) was frozen (−80°C) until bioassayed.

TIC bioassay

Ovarian theca-interstitial cells (TIC) were isolated from immature hypophysectomized rats as previously described (Magoffin & Erickson, 1988). 5×10^4 viable TIC were cultured (37°C) in 96-well tissue culture plates containing FCM (100 μl) diluted with an equal volume of fresh medium (total volume = 200 μl) for 2 days. The medium was frozen (−20°C) until assayed for androsterone by RIA (Zamecnik *et al.*, 1977).

Gel filtration chromatography

FCM was concentrated sixfold in a Speed Vac apparatus. 50 μl of concentrated FCM was filtered through a sterile 24.0 × 0.6 cm Biogel P-100 column equilibrated with Medium 199 containing 25 mM HEPES. 45 μl fractions were collected and assayed in the TIC bioassay. The void volume (V_0) was determined with blue dextran and the column was calibrated with albumin (66 000 M_r), carbonic anhydrase (29 000 M_r), cytochrome C (12 400 M_r) and aprotinin (6 500 M_r) by measuring the absorbance of the eluted proteins at 280 nm. A standard curve was generated by linear regression of M_r as a function of relative elution volume (Ve/Vo). The M_r of the bioactive substances was estimated by interpolating the Ve/Vo data on the standard curve.

Statistical analysis

Differences in bioactivity were determined by unpaired *t* test or, for multiple comparisons, by one way analysis of variance followed by Student-Newman-Keuls test. Significance was considered to be $P < 0.05$.

Acknowledgements

We would like to thank Dr. David MacLaughlin for providing the human MIS, Ms Sharon Carz for expert assistance with hormone assays and the National Hormone and Pituitary Program for providing ovine LH and recombinant human FSH.

References

Adashi, E.Y., Resnick, C.E., Tedeschi, C. & Rosenfeld, R.G. (1993). *Endocrinology*, **132**, 1463–1468.

Erickson, G.F., Li, D., Sadrkhanloo, R., Liu, X., Shimasaki, S. & Ling, N. (1994a). *Endocrinology*, **134**, 1365–1372.

Erickson, G.F., Magoffin, D.A., Dyer, C.A. & Hofeditz, C. (1985). *Endocr. Rev.*, **6**, 371–399.

Erickson, G.F., Mitchell, C., Nakatani, A., Ling, N. & Shimasaki, S. (1994b). *Endocrine*, **2**, 447–456.

Erickson, G.F., Nakatani, A., Ling, N. & Shimasaki, S. (1992a). *Endocrinology*, **130**, 1867–1878.

Erickson, G.F., Nakatani, A., Ling, N. & Shimasaki, S. (1992b). *Endocrinology*, **130**, 625–636.

Erickson, G.F., Nakatani, A., Liu, X., Shimasaki, S. & Ling, N. (1994c). *Molecular Biology of the Female Reproductive System*. Findlay, J.K. (ed), Academic Press: Orlando, pp. 101–127.

Erickson, G.F., Nakatani, A., Ling, N. & Shimasaki, S. (1993). *Endocrinology*, **133**, 1147–1157.

Hirshfield, A.N. (1991). *Biol. Reprod.*, **44**, 1157–1162.

Ingram, D.L. (1959). *J. Endocrinol.*, **19**, 117–122.

Khan, S.A., Keck, C., Gudermann, T. & Nieschlag, E. (1990). *Endocrinology*, **126,** 3043–3052.

Lischinsky, A. & Armstrong, D.T. (1982). *Can. J. Physiol. Pharmacol.*, **61,** 472–477.

Liu, X., Malkowski, M., Guo, Y., Erickson, G.F., Shimasaki, S. & Ling, N. (1993). *Endocrinology*, **132,** 1176–1183.

Magoffin, D.A. (1989). *Endocrinology*, **125,** 1464–1473.

Magoffin, D.A. & Erickson, G.F. (1982). *J. Biol. Chem.*, **257,** 4507–4513.

Magoffin, D.A. & Erickson, G.F. (1988). *Endocrinology*, **122,** 2345–2347.

Magoffin, D.A. & Erickson, G.F. (1994). *Molecular Biology of the Female Reproductive System.* Findlay, J.K. (ed.), Academic Press: Orlando, pp. 39–65.

Magoffin, D.A., Gelety, T.J. & Magarelli, P.C. (1994). *Ovulation Induction: Basic Science and Clinical Advances.* Filicori, M. & Flamigni, C. (eds), Elsevier Science Publishers: Amsterdam, pp. 25–36.

Magoffin, D.A., Kurtz, K.M. & Erickson, G.F. (1990). *Molec. Endocrinol.*, **4,** 489–496.

Magoffin, D.A. & Weitsman, S.R. (1993a). *Endocrinology*, **132,** 1945–1951.

Magoffin, D.A. & Weitsman, S.R. (1993b). *Biol. Reprod.*, **48,** 1166–1173.

Magoffin, D.A. & Weitsman, S.R. (1993c). *Molec. Cell. Endocrinol.*, **96,** 45–51.

Nakatani, A., Shimasaki, S., Erickson, G.F. & Ling, N. (1991). *Endocrinology*, **129,** 1521–1529.

Oliver, J.E., Aitman, T.J., Powell, J.F., Wilson, C.A. & Clayton, R.N. (1989). *Endocrinology*, **124,** 2671–2679.

Roy, S.K. & Greenwald, G.S. (1985). *Biol. Reprod.*, **32,** 203–215.

Sadrkhanloo, R., Hofeditz, C. & Erickson, G.F. (1987). *Endocrinology*, **120,** 146–155.

Zamecnik, J., Barbe, G., Moger, W.H. & Armstrong, D.T. (1977). *Steroids*, **30,** 679–689.