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Regulation of Expression of A-Kinase Anchoring Proteins in Rat Granulosa Cells[1]

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ABSTRACT

FSH action on granulosa cells involves the generation of cAMP and subsequent activation of the cAMP-dependent protein kinase (PKA). The PKA holoenzyme is targeted to specific subcellular sites through the interaction of the regulatory subunits with A-kinase anchoring proteins (AKAPs). We previously reported that FSH regulates expression of AKAPs. In this report we examine the relationship between AKAP expression and cell shape. Granulosa cells cultured in the absence of FSH tend to spread and flatten. Cell spreading is accompanied by an increased expression of a 140-kDa AKAP. This spreading/flattening phenotype is independent of the specific extracellular matrix proteins (fibronectin, polylysine, and gelatin) on which cells are plated. Addition of FSH prevents both cell spreading and induction of AKAP 140. Culturing cells on poly (2-hydroxyethyl methacrylate), a surface-coating agent that inhibits cell spreading and adhesion, also inhibits expression of AKAP 140. Addition of phorbol myristate acetate, an agent known to antagonize FSH actions, blocks FSH regulation of both cell shape and AKAP 140 expression. Addition of dexamethasone plus FSH causes a synergistic increase in progesterone levels but has no effect on cell shape or induction of AKAP 140. Dexamethasone produces a dose-dependent increase in AKAP 80 expression, which is blocked by FSH, suggesting cross talk between the glucocorticoid and FSH receptor signaling pathways. These data suggest that expression of AKAP 140 is linked to regulation of cell shape, and that changes in the expression of AKAPs are regulated by several different signaling pathways.

INTRODUCTION

The majority of the effects of FSH on ovarian granulosa cells are believed to be mediated via a mechanism involving the generation of cAMP and consequent activation of the cAMP-dependent protein kinase (PKA). Analogues of cAMP, or agents that raise intracellular levels of cAMP, mimic the effects of FSH to activate PKA [1–3]. The PKA holoenzyme consists of two regulatory (R) and two catalytic (C) subunits. Cyclic AMP binds to the R subunits, resulting in the dissociation and activation of the C subunits.

FSH stimulation of granulosa cell differentiation is characterized by several biochemical and morphological changes, including induction of the type IIβ regulatory subunit of PKA and a rearrangement of several cytoskeletal proteins, producing a rounded phenotype [1, 3–5]. These data suggest that PKA is an important mediator of FSH action; however, the mechanism by which PKA causes differentiation is still unclear. Our previous work suggests that the anchoring of PKA through its interaction with other cellular proteins referred to as A-kinase anchoring proteins (AKAPs) may be one factor regulating PKA actions in granulosa cells [6].

AKAPs are docking proteins that contain two classes of interacting domains: a high-affinity binding site for PKA subunits RIIα and/or RIIβ, and a second domain that targets PKA/AKAP complexes to specific intracellular sites [7–11]. It has been proposed that AKAPs target PKA to specific substrates or compartments within the cell, thus preventing inappropriate or random phosphorylation of other proteins. Previously, we identified the presence of several AKAPs in rat granulosa cells [6]. Characterization of these proteins as AKAPs was based both on their ability to bind RIIα and on the ability of the AKAP antagonist Ht31 to block binding of RIIα to each of the proteins [6]. While granulosa cells express a number of AKAPs, the expression of two AKAPs with molecular masses of 140 kDa and 80 kDa is regulated by FSH. The levels of AKAP 140 dramatically increase with time as granulosa cells are cultured on a fibronectin substratum; this increase is blocked if cells are treated with FSH [6]. FSH also blocks the flattening and spreading of cells on a fibronectin matrix [5], suggesting that AKAP 140 may be involved in regulating cellular adhesion to fibronectin. AKAP 140 is located predominantly in the particulate fraction [6] and therefore possibly associates with cytoskeletal or adhesion proteins.

In contrast with AKAP 140, addition of FSH to primary cultures of granulosa cells causes induction of AKAP 80 [6]. AKAP 80 is a cytosolic protein that preferentially binds to RIIα compared to RIIβ. FSH also causes a translocation of RIIα from the particulate to the cytosolic fraction, possibly as a result of increased expression of AKAP 80. One possible role for FSH-mediated PKA translocation may be to prepare the cells to be able to respond to LH, which also stimulates these cells via the cAMP second messenger pathway.

Although many AKAPs have been cloned and biochemically characterized, the physiological consequence of PKA/AKAP interaction remains unknown for most anchoring proteins [12–17]. To begin to understand the functional role of AKAPs in the hormonal regulation of granulosa cell differentiation, we have monitored the expression of AKAPs in response to FSH in the absence and presence of phorbol myristate acetate (PMA) or dexamethasone, agents that, respectively, antagonize or potentiate the effect of FSH. We have also monitored select markers of differentiation, including changes in cell shape and production of progesterone, to determine whether expression of specific AKAPs correlates with these physiological functions.

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MATERIALS AND METHODS

Materials

Ovine FSH (o-FSH-16) was obtained from NIADDK-NIH. PAGE reagents were obtained from Bio-Rad Laboratories (Hercules, CA), and culture medium was obtained from Life Technologies (Gaithersburg, MD). All other chemicals, including fibronectin, echistatin, and poly (HEMA), were obtained from Sigma Chemical Co. (St. Louis, MO).

Primary Granulosa Cell Cultures

Female Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) received s.c. injections of estradiol-17β (1.5 mg) in 0.1 ml propylene glycol daily at 24, 25, and 26 days of age to stimulate the development of preantral follicles [18]. On Day 28, rats were killed, the ovaries were removed, and granulosa cells were obtained from follicles by modifications to the method of Bley et al. [19]. The medium used for all procedures was Dulbecco's Modified Eagle's Medium-Ham's F-12 (DME:F-12 1:1), with 15 mM HEPES, 3.15 g glucose/L, 100 IU/ml penicillin-G, and 100 mg/ml streptomycin. Granulosa cells were extruded into the medium from individual follicles using 30-gauge needles and gentle pressure. Cells were pelleted at 100 × *g* for 15 min, counted using Trypan blue dye, and plated at a density of approximately 1×10^6 cells/ml on plastic dishes (Falcon Plastics, Los Angeles, CA) coated with 0.5 mg/ml human plasma fibronectin or, where indicated, other matrix proteins at the same concentration. In some cases, cells were preincubated with 100 nM echistatin for 30 min before being plated on fibronectin [20]. Cultured cells were grown in a humidified atmosphere at 37°C, 5% CO_2 with 10 nM estradiol-17β (in ethanol, final concentration 0.5%). FSH (50 ng/ml ovine FSH-16, diluted in medium) was added to individual plates where indicated. After incubation for 72 h, the medium was aspirated; and cells were rinsed with PBS, harvested by scraping in 1% SDS buffer containing 100 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS; pH 6.8) and 20% glycerol at 90°C, and then boiled for 5 min. Protein concentration was then determined using a BCA assay kit (Pierce, Rockford, IL). Samples were boiled again for 5 min after the addition of β-mercaptoethanol (5%). All animal studies were conducted in accord with the highest standards of humane animal care, as outlined in Guiding Principles for the Care and Use of Research Animals, promulgated by the Society for the Study of Reproduction.

Progesterone Assay

RIAs for progesterone were conducted by the Hormone and Neurotransmitter Core Facility (P30 Center for Research on Fertility and Infertility at Northwestern University) using a commercially available kit (ICN Biomedicals, Carson, CA).

RII Overlay Procedure

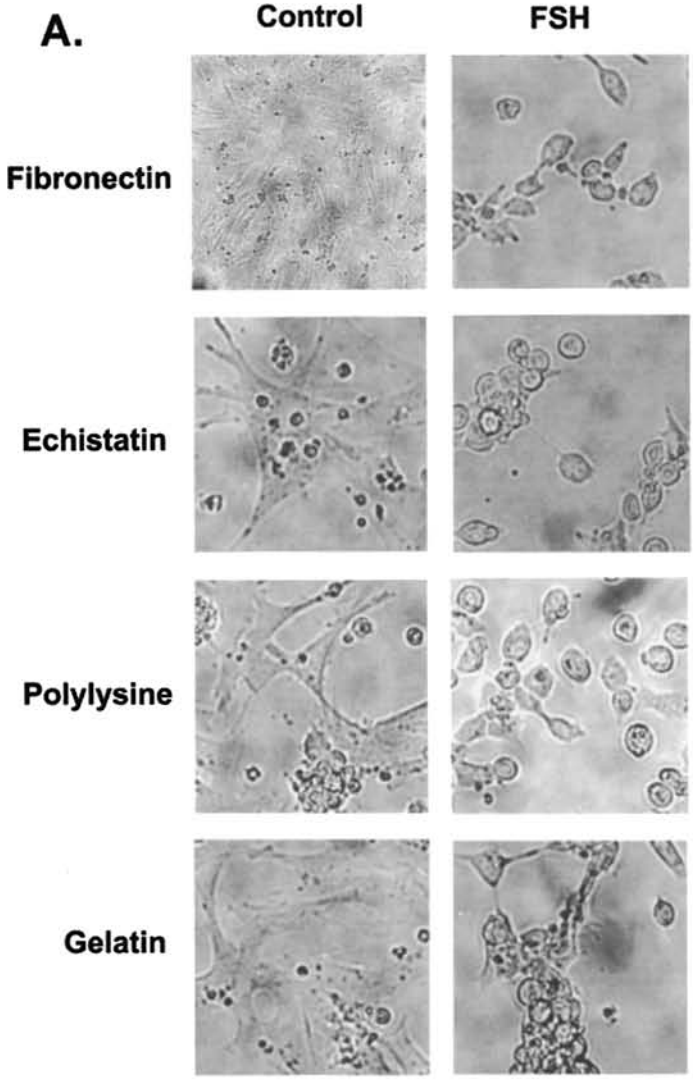
The overlay procedure is a modified Western blot procedure. Granulosa cells were harvested and solubilized as described above, and proteins were separated on SDS-PAGE and transferred to Immobilon (Millipore, Bedford, MA). Protein concentrations for each sample were determined using the BCA Protein Assay Kit, and equal amounts of protein (25 μg) were added to each lane. After incubation with Blotto (10 mM Tris-HCl [pH 7.5], 150 mM NaCl, 5% nonfat powdered milk, and 0.01% BSA) for 1 h to prevent nonspecific binding, the blot was incubated with radiolabeled RIIα [21]. Recombinant RIIα was produced and labeled as previously described [13, 22]. After separation from free [^{32}P]ATP, the ^{32}P-RII (500 000 cpm/10 ml blotto) was incubated with the blocked blot for 4 h, and washing and autoradiography followed. Statistical analysis of scanned autoradiographs were performed using Student's *t*-test.

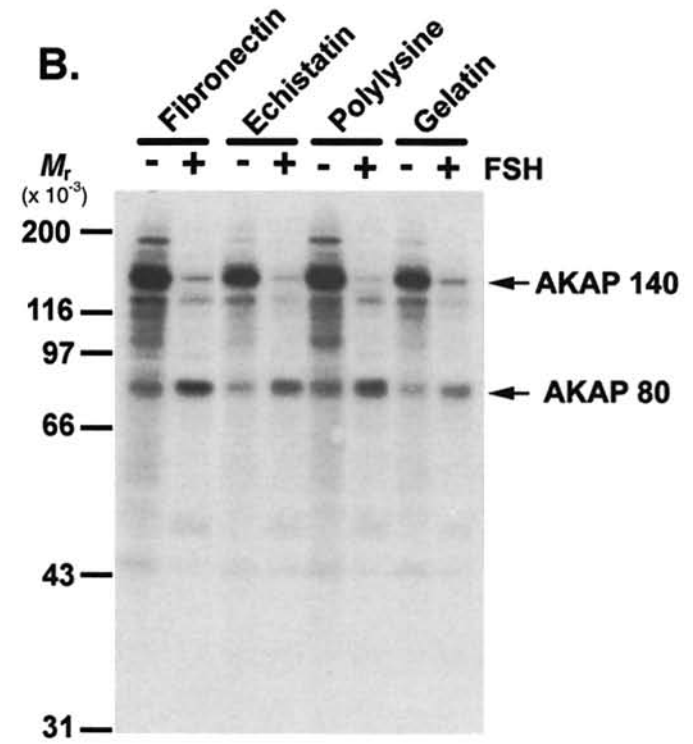
RESULTS

Effect of Extracellular Matrix on Cell Shape and AKAP Expression

We have previously observed that the spreading of granulosa cells grown on a fibronectin matrix is associated with a time-dependent increase in the production of AKAP 140 [6]. The addition of FSH and subsequent elevation of intracellular cAMP blocks this increase in AKAP 140. To determine whether the observed changes in cell morphology and AKAP 140 expression were dependent on the interaction of the cells with a specific extracellular matrix, we monitored cellular morphology and AKAP expression in granulosa cells cultured on a variety of different extracellular matrix proteins in the presence and absence of FSH (Fig. 1). Fibronectin, polylysine, or gelatin are common substrates used to support cell growth and are known to interact with different cellular adhesion molecules. Although the morphology of the cells was slightly different on each substrate, the FSH-treated cells consistently assumed a more rounded phenotype after 72 h of culture, whereas the untreated cells were spread out and flattened, and produced more cytoplasmic extensions (Fig. 1A). Preincubation of cells for 30 min with 100 nM echistatin, an RGD-rich peptide obtained from snake venom which inhibits binding of integrin receptors to immobilized fibronectin, also had no obvious effect on morphology. The relative patterns of expression of AKAPs 140 and 80 were dramatically altered by addition of FSH, whereas only comparatively minor changes were observed by changing extracellular matrix proteins (Fig. 1B). Even though there was a noticeable decrease in AKAPs treated with echistatin and gelatin, the significance of these changes is questionable because all the AKAPs in both the echistatin and gelatin lanes were decreased, suggesting that these changes may have been due to an artifact of preparation or loading. Although we do measure protein concentration and normalize each lane before loading, it is possible that the extracellular matrix proteins distorted our protein assay. Addition of FSH resulted in both a marked reduction in AKAP 140 (and other less prominent AKAPs) and an increase in AKAP 80. These results suggest that cellular interaction with immobilized extracellular matrix through RGD repeats is not required for cell spreading or production of AKAP 140. Indeed, even cells plated on uncoated plastic exhibited a flattened phenotype and produced high quantities of AKAP 140 (data not shown), suggesting that protein-protein interaction with preexisting extracellular matrix proteins is not required.

These results demonstrated that AKAP 140 was produced under conditions that promoted granulosa cell spreading. To determine whether cells cultured without FSH would produce AKAP 140 in the absence of spreading, we plated granulosa cells on poly (hydroxyethylmethacrylate) (poly[HEMA]), a surface coating agent that pre-





vents spreading and adhesion [23]. The mechanism by which poly(HEMA) reduces the adhesivity of polystyrene is not entirely known. Poly(HEMA) is a hydrophilic hydrogel of neutral charge. It may act by reducing the net negative electrostatic charge of the polystyrene, but other mechanisms are tenable. Coating the plates with poly(HEMA) prevented the granulosa cells from spreading and also blocked the production of AKAP 140 (Fig. 2, A and C), while control cells plated on fibronectin produced substantial amounts of AKAP 140 in the absence of FSH. These results are consistent with the hypothesis that AKAP 140 is induced only under conditions that promote cell spreading and adhesion. We considered the possibility that AKAP 140 may be an α5 integrin, which is also 140 kDa. However, Western analysis using a polyclonal antibody specific for α5 integrin detected a band, slightly higher than AKAP 140, that did not respond to FSH treatment (data not shown).

Phorbol Esters Antagonized Effects of FSH

Activators of protein kinase C (PKC) have been shown to antagonize several of the FSH-induced effects on granulosa cells. Addition of phorbol esters to granulosa cells blocks the FSH-induced increases in cAMP and progesterone production [24]. Activation of PKC is also known to promote cell spreading on fibronectin [25]. To determine the effects of phorbol esters on cellular morphology and production of AKAPs in granulosa cells, PMA was added to granulosa cells in the presence or absence of FSH (Fig. 3). Addition of 10 nM PMA had no effect on cell shape in the absence of FSH; however, PMA completely blocked the ability of FSH to produce a rounded phenotype (Fig. 3A) and blocked the ability of FSH to inhibit the expression of AKAP 140 (Fig. 3B). PMA also blocked the FSH-induced increases in AKAP 80 and progesterone. Once again, the association between increased AKAP 140 expression and a nonrounded phenotype was confirmed.

Dexamethasone Stimulated Expression of AKAP 80

Dexamethasone synergizes with FSH to induce progesterone production in granulosa cells [26, 27]. Dexamethasone was added to granulosa cells plated on polylysine (over a concentration range of 1–1000 nM), and cells were monitored for altered cell shape, AKAP expression, and progesterone production (Figs. 4 and 5). As previously described [26, 27], addition of dexamethasone by itself had little effect on progesterone production but, when added in combination with FSH, produced a synergistic increase in medium progesterone levels (data not shown). Treatment of cells with dexamethasone in the presence or absence of FSH did not produce a notable effect on the morphology of the cells (Fig. 4A). Although there appeared to be an increase in AKAP 140 expression at 1 and 10 nM dexamethasone in some experiments (Fig. 4B), statistical analysis of densitometric scans from 5 different experiments did

FIG. 1. Effect of extracellular matrix proteins on granulosa cell shape and AKAP expression. Immature granulosa cells were cultured with or without FSH (50 ng/ml) on plates coated with fibronectin, fibronectin plus echistatin, polylysine, or gelatin, as described in *Materials and Methods*. **A**) Photomicrographs (×320; reproduced at 81%) were taken after 72-h incubation at 37°C. **B**) The same cells were then harvested and analyzed for AKAP expression by RII overlay assay. These results are representative of two separate experiments.

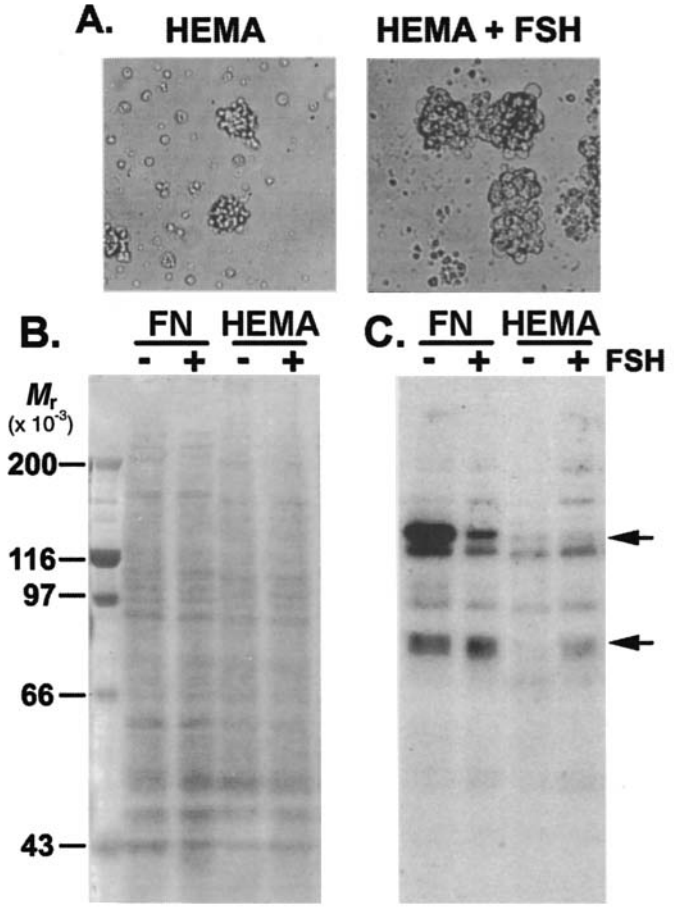


FIG. 2. Effect of poly(HEMA) on granulosa cell shape and AKAP expression. Immature granulosa cells were cultured with or without FSH (50 ng/ml) on plates coated with poly(HEMA), 12 mg/60-mm dish. **A**) Photomicrographs (×200, reproduced at 80%) were taken after 72-h incubation at 37°C. The same cells, plus control cells that had been plated on fibronectin (FN), similar to those pictured in Figure 1, were then harvested and stained with Coomassie brilliant blue dye (**B**) or analyzed for AKAP expression by RII overlay assay (**C**). The arrows identify AKAPs 140 and 80. These results are representative of three separate experiments.

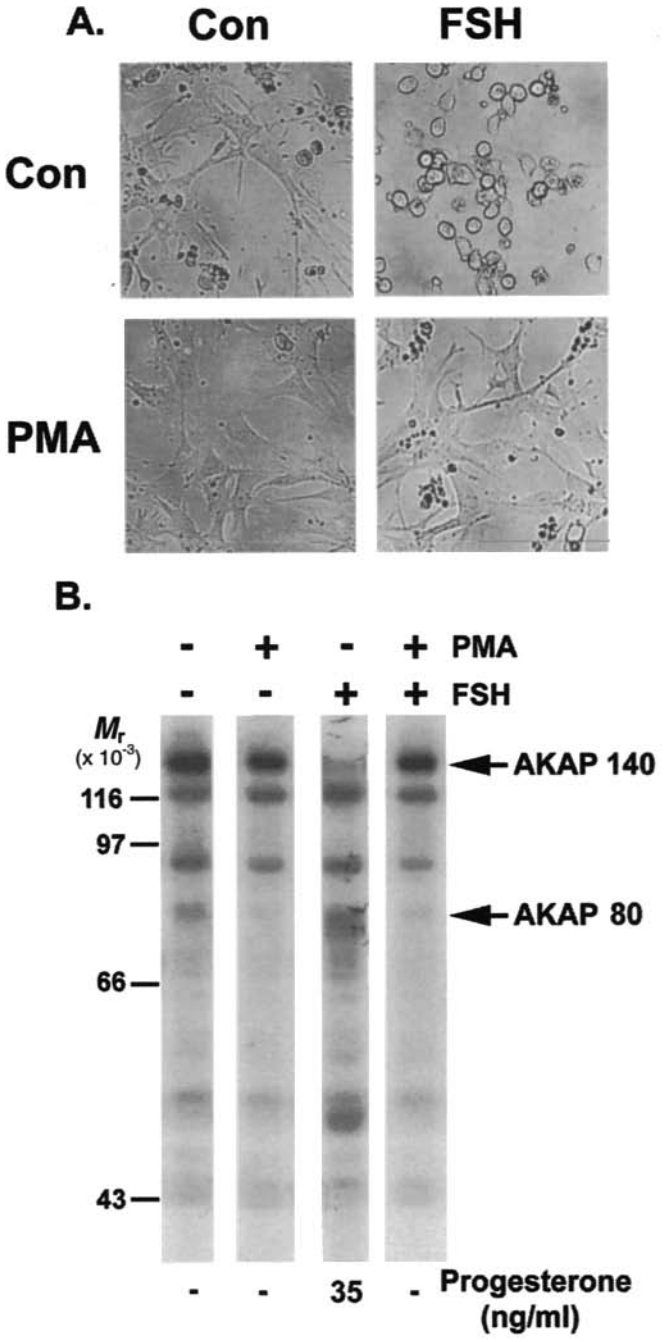


FIG. 3. Effect of PMA on granulosa cell shape and AKAP expression. Immature granulosa cells were cultured with or without FSH (50 ng/ml) and PMA (10 nM). **A**) Photomicrographs (×200, reproduced at 80%) were taken after 72-h incubation at 37°C. **B**) The same cells were then harvested and analyzed for AKAP expression by RII overlay assay. Media progesterone levels are shown at the bottom of the figure. The dashes indicate below-detectable levels (< 0.1 ng/ml). These results are representative of three separate experiments.

not corroborate a significant change. However, dexamethasone, in a concentration-dependent manner, dramatically increased AKAP 80 expression to a level much higher than that seen with FSH treatment alone (Figs. 4B and 5). Surprisingly, even though FSH caused an increase in the expression of AKAP 80 compared to levels in untreated control granulosa cells (compare lanes 1 and 6), FSH blocked the ability of dexamethasone to increase expression of AKAP 80 (compare lanes 4,5 with 9,10). This result suggests that treatment of the cells with FSH interferes with a signaling pathway necessary for dexamethasone to increase expression of AKAP 80. Even though FSH and dexamethasone both stimulated an increase of AKAP 80, FSH but not dexamethasone had an inhibitory effect on production of AKAP 140, suggesting that alternate pathways regulate the production of these two AKAPs.

DISCUSSION

It has been well established that when immature granulosa cells are cultured in serum-free medium in the absence of added FSH or growth factors, they assume a flattened epithelioid phenotype, characterized by the presence of extensive cytoplasmic extensions. Treatment with FSH causes these cells to assume a nearly spherical shape. This finding correlates with the previously described transformation from flattened to cuboid granulosa cells that occurs during folliculogenesis [28]. These FSH-stimulated changes in cell shape involve a rearrangement of the cytoskeleton including down-regulation of the expression of actin and actin-binding proteins and reorganization of thin filaments and microtubules [29]. Differentiation of granulosa cells also involves changes in cell contact and intercellular communication molecules. Adherence junctions and desmosomes

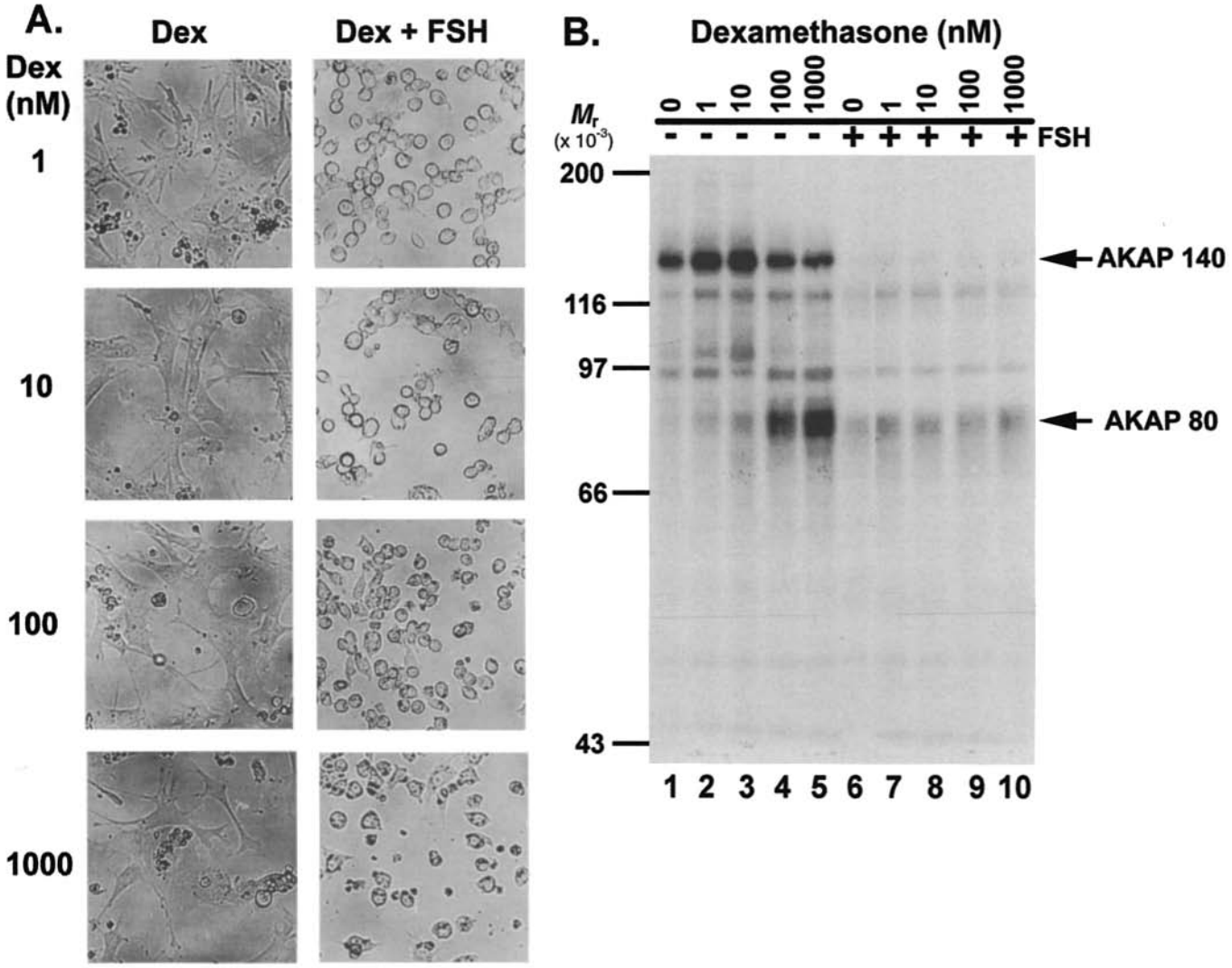


FIG. 4. Effect of dexamethasone on granulosa cell shape and AKAP expression. Immature granulosa cells were cultured with or without FSH (50 ng/ml) in addition to increasing concentrations of dexamethasone (Dex). **A**) Photomicrographs (×200) were taken after 72-h incubation at 37°C. **B**) The same cells were then harvested and analyzed for AKAP expression by RII overlay assay.

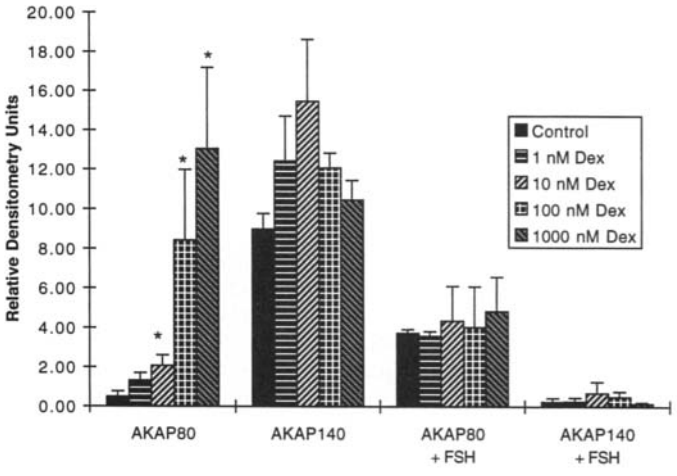


FIG. 5. Quantification of AKAP 80 and 140 by densitometry. *Significant ($p < 0.05$) difference from control.

are down-regulated, whereas gap junctions are increased in number and in size [29].

In this report we have shown that granulosa cell shape changes are accompanied by a change in the production of AKAP 140. In the absence of FSH, production of AKAP 140 increases in a time-dependent manner over a 3-day culture period [6]. Increased AKAP 140 expression was associated with cellular spreading and cytoplasmic extensions. Spreading of granulosa cells did not appear to be induced by interaction with specific extracellular matrix proteins, since spreading was qualitatively equivalent when cells were cultured on three different matrix proteins or on plastic alone. Although the extent of spreading was altered somewhat with the various matrix proteins and by the addition of echistatin, a peptide that contains several RGD motifs and therefore should compete with the solid-phase fibronectin, under all conditions the cells were flattened. Addition of FSH blocked both the induction of AKAP 140 [6] and cell spreading. Cell spreading and induction of AKAP 140 was also blocked by coating the plates with poly(HEMA), a surface-coating polymer used to prevent adhesion of cells to polystyrene. Treatment of cells with 10 nM PMA antagonized the ability of FSH to cause cellular

rounding and prevented the FSH-mediated decrease of AKAP 140 expression. These results demonstrate that AKAP 140 is produced under conditions that encourage granulosa cell spreading and suggest that AKAP 140 is involved in the regulation of cell shape and/or cytoskeletal rearrangement.

Steroid hormones have been shown to enhance gonadotropin stimulation of ovarian granulosa cell differentiation. Dexamethasone is a glucocorticoid that can act both synergistically or antagonistically with FSH. Cotreatment of granulosa cells with FSH and dexamethasone (over a range of 0.01 to 1 μM) synergistically enhanced the production of progesterone [26, 27] and the secretion of tissue-type plasminogen activator [30]. Dexamethasone also potentiates insulin-like growth factor (IGF)-I stimulation of cytochrome P450 enzyme, and this potentiation is blocked by RU-486 [31]. However, dexamethasone (1 μM) inhibits FSH-stimulated induction of LH/hCG receptors and aromatase activity [26]. Addition of dexamethasone to granulosa cells produced a striking (26-fold) dose-dependent increase in AKAP 80 but had little or no effect on expression of AKAP 140 or on cell shape. These are the only experimental conditions under which we have detected simultaneous expression of relatively high levels of both AKAPs 80 and 140. The levels of AKAP 80 expression induced by dexamethasone were considerably higher than those induced by FSH. When cells were cultured with FSH plus dexamethasone, the hyper-expression of AKAP 80 seen with dexamethasone alone was reduced to levels equivalent to those induced by FSH alone. That expression levels of AKAP 80 and 140 in cells grown with FSH and dexamethasone reflect a typical FSH-stimulated granulosa cell phenotype suggests that FSH inhibits the dexamethasone-stimulated AKAP 80 expression. The fact that FSH dominates over dexamethasone in regulating AKAP expression suggests that neither the synergistic effect of dexamethasone plus FSH on progesterone production nor the antagonistic effects of dexamethasone on induction of LH/CG receptors or aromatase activity are related to large changes in AKAP 80 or 140 expression.

AKAPs function to target type II PKAs to specific subcellular locations. AKAPs can also bind other enzymes, including protein kinase C [32, 33] and protein phosphatase 2B [34], presumably to regulate the phosphorylation of one or more substrate proteins through various signaling pathways [10]. Some AKAPs, such as graven, are associated with cytoskeletal proteins and are thought to play a role in adherent processes [33]. Very little is known about what regulates the expression of AKAPs. Our data suggest that the expression of AKAPs 80 and 140 are uniquely regulated by stimulation of both membrane and steroid receptors and by both PKA and PKC second messenger pathways. Future studies will be directed towards identifying and characterizing AKAPs 80 and 140 and determining whether they associate with enzymes or cellular proteins in addition to PKA.

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REFERENCES

1. Hsueh AJW, Adashi EY, Jones PBC, Welsh THJ. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr Rev 1984; 5:76–127.
2. Richards JS, Jahnsen T, Hedin L, Lifka J, Ratoosh S, Durica JM, Goldring NB. Ovarian follicular development: from physiology to molecular biology. Recent Prog Horm Res 1987; 43:231–276.
3. Richards JS, Hedin L. Molecular aspects of hormone action in ovarian follicular development, ovulation, and luteinization. Annu Rev Physiol 1988; 50:441–463.
4. Hsueh AJW, Bicsak TA, Jai XC, Dahl KD, Fauser BCJM, Galway AB, Czekala N, Pavlou SN, Papkoff H, Keene J, Boime I. Granulosa cells as hormone targets: role of biologically active FSH in reproduction. Recent Prog Horm Res 1989; 45:209–274.
5. Amsterdam A, Rotmensch S. Structure-function relationships during granulosa cell differentiation. Endocr Rev 1987; 8:309–337.
6. Carr DW, DeManno DA, Atwood A, Hunzicker-Dunn M, Scott JD. Follicle-stimulating hormone regulation of A-kinase anchoring proteins in granulosa cells. J Biol Chem 1993; 268:20729–20732.
7. Scott JD, Carr DW. Subcellular localization of the type II cAMP-dependent protein kinase. News Physiol Sci 1992; 7:143–148.
8. Scott JD, McCartney S. Localization of A-kinase through anchoring proteins. Mol Endocrinol 1994; 8:5–11.
9. Rubin CS. A kinase anchor proteins and the intracellular targeting of signals carried by cyclic AMP. Biochim Biophys Acta 1994; 1224: 467–479.
10. Faux MC, Scott JD. Molecular glue: kinase anchoring and scaffold proteins. Cell 1996; 85:9–12.
11. Dell'Acqua ML, Scott JD. Protein kinase A anchoring. J Biol Chem 1997; 272:12881–12884.
12. Bregman DB, Hirsch AH, Rubin CS. Molecular characterization of bovine brain P75, a high affinity binding protein for the regulatory subunit of cAMP-dependent protein kinase II beta. J Biol Chem 1991; 266:7207–7213.
13. Carr DW, Hausken ZE, Fraser ID, Stofko-Hahn RE, Scott JD. Association of the type II cAMP-dependent protein kinase with a human thyroid RII-anchoring protein. Cloning and characterization of the RII-binding domain. J Biol Chem 1992; 267:13376–13382.
14. Carr DW, Stofko-Hahn RE, Fraser ID, Cone RD, Scott JD. Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins. Characterization of AKAP 79. J Biol Chem 1992; 267:16816–16823.
15. Coghlan VM, Langeberg LK, Fernandez A, Lamb NJ, Scott JD. Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. J Biol Chem 1994; 269:7658–7665.
16. Lin R, Moss SB, Rubin CS. Characterization of S-AKAP84, a novel developmentally regulated A kinase anchor protein of male germ cells. J Biol Chem 1995; 270:27804–27811.
17. McCartney S, Little BM, Langeberg LK, Scott JD. Cloning and characterization of A-kinase anchor protein 100 (AKAP100). J Biol Chem 1995; 270:9327–9333.
18. Ratoosh SL, Lifka J, Hedin L, Jahsen T, Richards JS. Hormonal regulation of the synthesis and mRNA content of the regulatory subunit of cyclic AMP-dependent protein kinase type II in cultured rat ovarian granulosa cells. J Biol Chem 1987; 262:7306–7313.
19. Bley MA, Simon JC, Saragueta PW, Baranau JL. Hormonal regulation of rat granulosa cell deoxyribonucleic acid synthesis: effect of estrogens. Biol Reprod 1991; 44:880–888.
20. Karczewski J, Endris R, Connolly TM. Disagregin is a fibrinogen receptor antagonist lacking the Arg-Gly-Asp sequence from the tick, *Ornithodoros moubata*. J Biol Chem 1994; 269:6702–6708.
21. Carr DW, Scott JD. Blotting and band-shifting: techniques for studying protein-protein interactions. Trends Biochem Sci 1992; 17:246–249.
22. Carr DW, Stofko-Hahn RE, Fraser ID, Bishop SM, Acott TS, Brennan RG, Scott JD. Interaction of the regulatory subunit (RII) of cAMP-dependent protein kinase with RII-anchoring proteins occurs through an amphipathic helix binding motif. J Biol Chem 1991; 266:14188–14192.
23. Folkman J, Moscona A. Role of cell shape in growth control. Nature 1978; 273:345–349.
24. Trzeciak WH, Duda T, Waterman MR, Simpson ER. Tetradecanoyl phorbol acetate suppresses follicle-stimulating hormone-induced synthesis of the cholesterol side-chain cleavage enzyme complex in rat ovarian granulosa cells. J Biol Chem 1987; 262:15246–15250.
25. Vuori K, Ruoslahti E. Activation of protein kinase C precedes a5b1 integrin-mediated cell spreading on fibronectin. J Biol Chem 1993; 268:21459–21462.
26. Schoonmaker JN, Erickson GF. Glucocorticoid modulation of follicle-

stimulating hormone-mediated granulosa cell differentiation. Endocrinology 1983; 113:1356–1363.
27. Adashi EY, Jones PBC, Hsueh AJW. Synergistic effect of glucocorticoids on the stimulation of progesterone production by follicle-stimulating hormone in cultured rat granulosa cells. Endocrinology 1981; 109:1888–1894.
28. Cormack DH, Ham's Histology. Ninth ed. New York: J.B. Lippincott Company; 1987.
29. Amsterdam A, Plehn-Dujowich D, Suh BS. Structure-function relationships during differentiation of normal and oncogene-transformed granulosa cells. Biol Reprod 1992; 46:513–522.
30. Jia XC, Ny T, Hsueh AJ. Synergistic effect of glucocorticoids and androgens on the hormonal induction of tissue plasminogen activator activity and messenger ribonucleic acid levels in granulosa cells. Mol Cell Endocrinol 1990; 68:143–151.
31. Urban RJ, Bodenburg YH, Nagamani M, Peirce J. Dexamethasone potentiates IGF-I actions in porcine granulosa cells. Am J Physiol 1994; 267:E115–123.
32. Klauck TM, Faux MC, Labudda K, Langeberg LK, Jaken S, Scott JD. Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. Science 1996; 271:1589–1592.
33. Nauert JB, Klauck TM, Langeberg LK, Scott JD. Graven, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein. Curr Biol 1996; 7:52–62.
34. Coghlan VM, Perrino BA, Howard M, Langeberg LK, Hicks JB, Gallatin WM, Scott JD. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. Science 1995; 267: 108–111.