Dexamethasone Enhances Follicle Stimulating Hormone-Induced P450scc mRNA Expression and Progesterone Production in Pig Granulosa Cells

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Abstract

The effect of dexamethasone on follicle-stimulating hormone (FSH)-stimulated expression of cholesterol side-chain cleavage (P450scc) enzyme and production of progesterone by ovarian granulosa cells was studied in vitro. Granulosa cells from 3- to 5-mm pig antral follicles were cultured for 48 h in the presence or absence of FSH and/or dexamethasone. Treatment with FSH resulted in a dose-dependent increase in the level of P450scc mRNA that reached a submaximum at 100 ng FSH/ml. This increase was associated with an increase in progesterone production. Treatment of the cells with increasing concentrations (10^{-9} – 10^{-6} M) of dexamethasone for 48 h increased constitutive and potentiated FSH-stimulated P450scc mRNA levels and progesterone production in a dose-dependent manner. Increasing duration (12-48 h) of treatment with dexamethasone (100 nM) led to a time-dependent increase in basal and FSH-stimulated progesteorne production, achieving statistical significance by 48 and 24 h, respectively. Dexamethasone also increased P450scc mRNA level and progesterone production induced by the adenylate cyclase activator forskolin (10 μM) or a cAMP analog 8-Br-cAMP (1 mM). The effects of dexamethasone on FSH-induced progesterone production were blocked by cotreatment of the cells with glucocorticoid receptor antagonist RU-486. These results demonstrate that dexamethasone potentiates FSH actions on steroidoogenesis in the pig ovary. Possible mechanisms for this potentiation include the ability of dexamethasone to stimulate P450scc gene expression.

Key Words: pig granulosa cells, progesterone, dexamethasone, P450scc, RU-486

Introduction

The biosynthesis of gonadal steroid hormones – progesterone, estrogens, and androgens – begins from cholesterol. Cellular cholesterol is acquired principally from blood-borne lipoproteins, which associate with specific cell-membrane receptors to facilitate sterol uptake, utilization, and/or storage, e.g. as cholesteryl esters in cytoplasmic lipid droplets (19). Tropic hormones can control the cellular uptake and/or intracellular liberation of free sterol, and facilitate its transport from extramitochondrial sites to the inner mitochondrial membrane, where the cytochrome P450 side-chain cleavage (P450scc) enzyme cleaves cholesterol into pregnenolone (42). Cytochrome P450scc is encoded by the CYP11A gene (30). Recent observations corroborate earlier notions that free sterol delivery and access to the inner mitochondrial P450scc hydroxylation reaction is a rate-limiting and a pivotal locus of hormonal control. This transfer process is

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mediated in significant part by the cycloheximide-sensitive hormonally inducible protein, steroidogenic acute regulatory (StAR) protein (2, 8).

Glucocorticoids are hormonal mediators of stress. They potentially affect normal gonadal function by acting at any one or more of the following levels in hypothalamic-pituitary-gonadal axis: (i) the hypothalamus (to decrease the synthesis and release of GnRH); (ii) the anterior pituitary gland (to inhibit the synthesis and release of gonadotropins); (iii) the testis/ovary (to modulate steroidogenesis and/or gametogenesis directly). It was thought that the principal deleterious actions of the glucocorticoids occurs at the hypothalamus and the anterior pituitary gland, creating a state of hypogonadotropic hypogonadism. This view is supported by a large body of data including observations that administration of synthetic glucocorticoids can significantly decrease hypothalamic GnRH release (10, 14, 34) and can inhibit the GnRH-stimulated release of LH and FSH from the pituitary (4, 25, 26, 28, 34). However, recent studies have identified glucocorticoid receptors in ovarian and testicular cells (11, 23, 35, 36) and have clearly shown that glucocorticoids have direct actions on gonadal steroidogenesis, both in vivo and in vitro (3, 11, 15, 17, 27, 29, 43). Another mechanism by which the hypothalamic-pituitary-adrenal axis may influence reproductive function is by a direct effect of glucocorticoids on the target tissues of sex steroids (32).

It has been reported that in rat and human granulosa cells that have not luteinized in vitro, glucocorticoids can potentiate the cAMP and steroid responses to LH and FSH (1, 3, 13, 16, 17), whereas in human granulosa-lutein cells, glucocorticoids inhibit LH-stimulated pregnenolone production (29). This would suggest that the effects of glucocorticoids on the steroidogenic activity of a given ovarian cell type may vary with the stage of the ovarian cycle and the differentiation state of the cell; i.e. glucocorticoids may enhance FSH action in granulosa cells in the follicular phase of the ovarian cycle but predominantly attenuate LH-stimulated steroidogenesis during the luteal phase. To test this hypothesis, we have recently used rat preovulatory follicles to demonstrate the effect of dexamethasone to suppress LH-stimulated progesterone production (18). The granulosa cells from preovulatory follicles of the adult cycling rat might be considered more mature and highly differentiated. The present work was undertaken to further study the mechanism of action of glucocorticoids on basal and

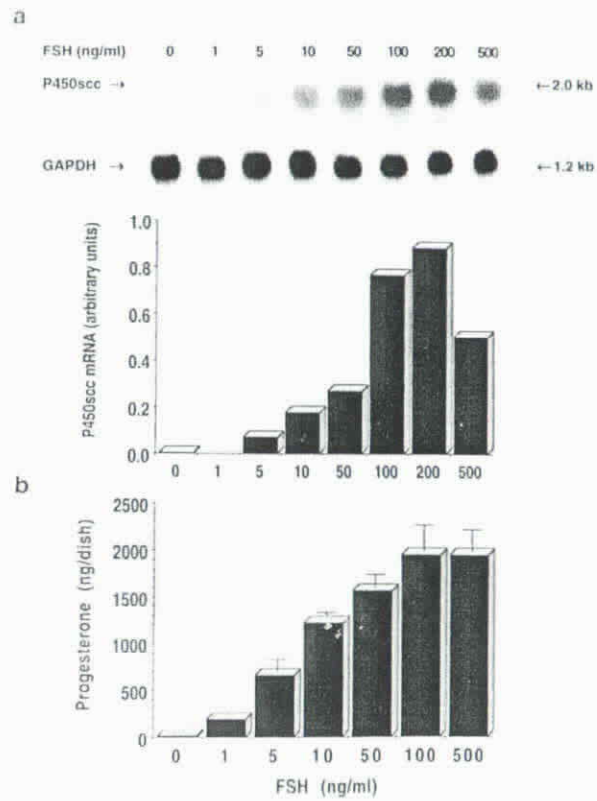

Fig. 1. Dose-response effects of FSH on P450scc mRNA expression (a) and progesterone production (b). Pig granulosa cells were cultured as described in *Materials and Methods* and at 48-h of the culture the medium was removed and replaced with medium minus serum. Except in the case of control cells, FSH was added to the dishes at concentrations ranging from 1 to 500 ng/ml. At 48 h, RNA was extracted from the incubated cells. Samples of total RNA (25 μg) were subjected to agarose gel electrophoresis, blotted onto nylon membrane, and hybridized with [^{32}P] dCTP-labelled cDNA encoding human $P450_{scc}$. Hybridization obtained with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as a loading control. The position of the P450scc and GAPDH mRNAs are indicated. Autoradiographs were quantitated; integrated optical density values were corrected for the amount of GAPDH in each lane and presented as arbitrary units. The data represent the results from a single experiment that was performed at least three times with similar results. The progesterone concentration was determined by RIA in duplicate samples from two culture dishes at each dose of FSH. Values are the mean ± SEM from four independent experiments. $P < 0.05$ between FSH = 0 and FSH = 1 ng/ml or above

FSH-stimulated progesterone production by pig granulosa cells that have not luteinized in vitro. The effects of glucocorticoids on the expression of P450scc gene were also tested.

Materials and Methods

Materials

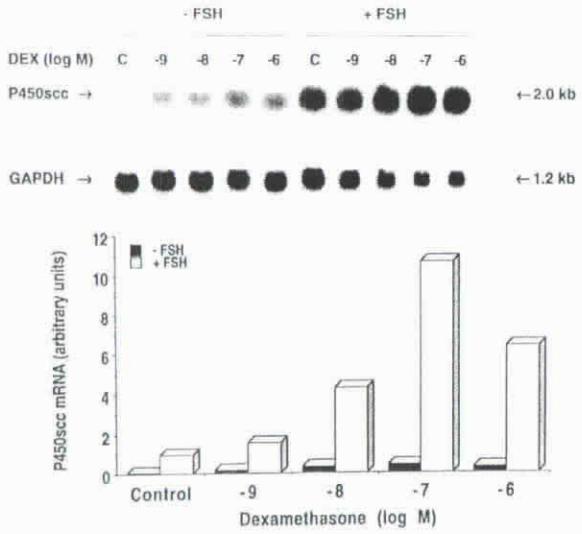

Fig. 2. Effect of the dose of dexamethasone (DEX) on FSH-stimulated P450scc mRNA expression. The conditions for this experiment are identical to those described for Figure 1 with the exception that the cells were cultured with or without (C, control) FSH (100 ng/ml) in the presence or absence of increasing concentrations of DEX (10^{-9} – 10^{-6} M). The Northern blots were analyzed for P450scc mRNA. After quantitation, integrated optical density values were corrected for the amount of GAPDH in each lane and presented as arbitrary units. The data represent the results from a single experiment that was performed at least three times with similar results.

Purified porcine pituitary FSH (USDA-pFSH-I1) was obtained from the National Hormone and Pituitary Distribution Program (Bethesda, MD, USA). Dexamethasone (dexamethasone phosphate) was obtained from Narn Guang Chemical Co. (Tainan, Taiwan). RU-486 (Mifepristone) was provided by Roussel-Uclaf (Paris, France). Ham's F12/Dulbecco's modified Eagle's medium (F12/DMEM; 1:1), horse serum, fetal calf serum (FCS) and other culture supplies were purchased from Gibco-BRL (Grand Island, NY, USA). Forskolin, 8-bromo-cyclic adenosine 3',5'-monophosphate (8-Br-cAMP) and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation and Culture of Granulosa Cells

Ovaries collected from freshly slaughtered pigs at a local slaughterhouse were carried to the laboratory in chilled HEPES buffer (137 mM NaCl, 5 mM KCl, 0.7 mM $NaHPO_4$, 25 mM HEPES, 10 mM glucose and 360 μM $CaCl_2$) containing 0.1% BSA, penicillin (100 U/ml) and streptomycin sulfate (200 μg/ml). Granulosa cells were isolated from 3- to 5-mm antral follicles by aspiration with a 26 gauge needle. The cells were centrifuged, washed, resuspended, counted in a hemacytometer, and allowed to attach to culture dishes in F12/DMEM supplemented with L-glutamine (2 mM), pecicillin (100 U/ml), streptomycin sulfate (100 μg/ml), fungizone (0.625 μg/ml), horse serum (12.5%), and FCS (2.5%) at a concentration of 20 x 10^6 viable cells/10-cm dish for 48 h at 37°C in a 5% CO_2-air atmosphere. The two sera were pretreated with dextran-charcoal (9). Before being used in an experiment, the cell cultures were rinsed twice with phosphate-buffered saline (PBS). Cells were then incubated in 4 ml F12/DMEM (without serum) containing test substances for 48 h or as indicated. Media were collected after the incubation and stored at -20°C until assayed for progesterone by radioimmunoassay (RIA) and the cells were harvested for RNA extraction. The percentage of cells plated that eventually attached to the dishes was not determined. Data in this study were obtained from 26 preparations of cells, each being prepared from 50 ovaries. Within each preparation, there were two replicates per treatment.

RNA Isolation and Northern Blotting

Total RNA was recovered by phenolic extraction in the presence of guanidinium thiocyanate, using a simplified version of a previously described method by Chomczynski and Sacchi (7). Cells were washed in cold PBS solution before recovery (at room temperature) into 2 ml TRISOLV solution (Cinna Biotecx, Houston, TX) and transfer to two microfuge tubes (1 ml/tube). Phase separation was then achieved by the addition of 0.2 ml chloroform per 1 ml of the TRISOLV, standing on ice for 2 min, and centrifugation (12,000*g*; 15 min; 4°C). The upper phase (0.7 ml) was transferred to a second microfuge tube, and RNA was precipitated by the addition of 0.7 ml isopropanol per 1 ml of the TRISOLV used for homogenization and standing on ice for 10 min. RNA was recovered by centrifugation (12,000*g*; 10 min; 4°C) and washed once in 100% ethanol (0.5 ml). The RNA pellet was then stored in 100% ethanol (0.5 ml) at -85°C until Northern blot analysis. Before analysis, samples were centrifuged for 5 min at 12,000*g* at 4°C. The pellet was resuspended in 75% ethanol (1 ml), and then reprecipitated by centrifugation (12,000*g*; 5 min; 4°C). The RNA pellet was dissolved in 6-10 μl of Milli Q water and quantitated by reading the absorbance at 260 nm.

The same amount of RNA sample (25 μg) was denatured in a mixture of glyoxal (1 M) and sodium

Table 1. Effect Of Dexamethasone (DEX) On Forskolin-Stimulated Progesterone Production By Cultured Pig Granulosa Cells[a]

	Progesterone (ng/dish)	
Forskolin (M)	- DEX	+ DEX
0	8.68 ± 0.79	14.59 ± 1.15[b]
10^{-7}	17.58 ± 2.72	39.50 ± 6.28[b]
10^{-6}	195.03 ± 35.39	260.90 ± 72.31[b]
10^{-5}	1065.30 ± 117.91	1389.97 ± 41.30[b]

[a]Granulosa cells were cultured with 100 nM DEX (+ DEX) or without DEX (- DEX) in the presence or absence of increasing concentrations (10^{-7} – 10^{-5} M) of forskolin. The progesterone concentration was measured in duplicate samples from two culture dishes at each dose of forskolin after 48-h incubations. Values are the mean ± SEM from four independent experiments. Significance of difference between + DEX and – DEX for each dose of forskolin: [b]$P < 0.05$.

phosphate (10 mM; pH 6.5) for 60 min at 50°C, then size-fractionated by electrophoresis gels containing 1 % agarose using sodium phosphate (10 mM; pH 6.5) as the running buffer and transferred to Hybond-N nylon membranes (Amersham Pharmacia Biotech, Taipei, Taiwan) by pressure blotting (50-60 mbar; 90 min; VacuGene XL Blotting Unit, Pharmacia LKB Biotechnology AB, Uppsala, Sweden) (38). RNA was cross-linked to nylon membrane with UV light using a UV Stratalinker 1800 (Stratagene, La Jolla, CA). The migration position of ribosomal RNA (28S and 18S) was determined by 0.02% methylene blue (in 0.5 M sodium acetate; pH 4.8) staining.

cDNA Labelling and Hybridization Analysis

Human P450scc and mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs were used as probes. P450scc cDNA was a generous gift from Dr. Bon-Chu Chung (Institute of Molecular Biology, Academia Sinica, Nankang, Taiwan). Mouse GAPDH cDNA was a gift from Dr. Hsiao-Sheng Liu (Department of Microbiology, National Cheng Kung University Medical College, Tainan, Taiwan). Plasmid preparation of the two different cDNA inserts was carried out using QIAGEN anion-exchange columns (QIAGEN protocol; DIAGEN GmbH, Dusseldorf, Germany). The cDNA inserts were removed from the plasmids by digestion with restriction endonucleases. The respective cDNA inserts were purified by electrophoresis in 1% agarose gel after digestion with appropriate restriction enzymes. The specific cDNAs were labelled with [α-^{32}P] deoxy-CTP (Specific activity = 3000 Ci/mmol; Amersham) to a specific activity of 10^8 to 10^9 c.p.m./μg using the

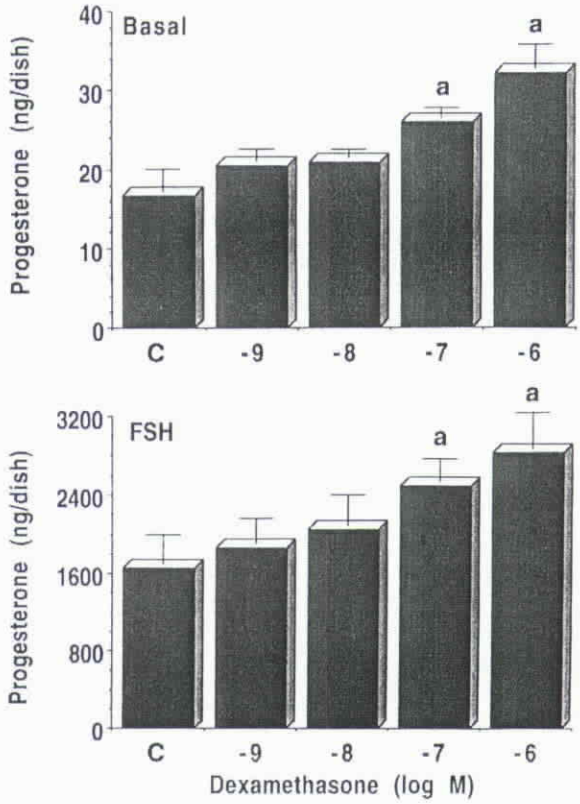

Fig. 3. Effect of the dose of dexamethasone on FSH-stimulated progesterone production. Granulosa cells were cultured with or without (basal) FSH (100 ng/ml) in the presence or absence (C, control) of increasing concentrations (10^{-9} – 10^{-6} M) of dexamethasone. The progesterone concentration was determined in duplicate samples from two culture dishes at each dose of dexamethasone. Values are the mean ± SEM from three independent experiments. [a]Significantly different from the corresponding control group in which no dexamethasone was added; $P < 0.05$.

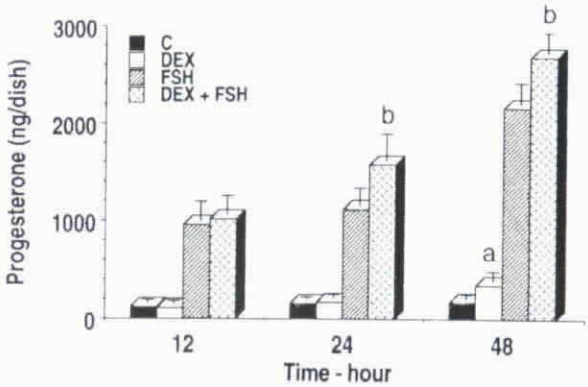

Fig. 4. Time-course of dexamethasone (DEX) stimulation of progesterone production. Granulosa cells were cultured in the absence (C, control) or presence of FSH (100 ng/ml) with or without DEX (100 nM) at the designated time points. Media were collected and the progesterone released into the medium was measured in duplicate samples from two culture dishes at each time point. Values are the mean ± SEM from three independent experiments. [a]Significantly different from the corresponding time point of the control group, $P < 0.05$; [b]significantly different from the corresponding time point of the FSH group, $P < 0.05$.

Megaprime DNA Labelling System (Amersham) (12). The labelled cDNAs were separated from free radionucleotides by Sephadex G-50 (Fine; Pharmacia LKB Biotechnology AB) chromatography.

The membranes were prehybridized (1 h) at 60°C with shaking in QuikHyb solution (Stratagene). Hybridization was carried out for 3 h at 60°C in the same solution containing heat-denatured [^{32}P]-labelled cDNAs and salmon sperm DNA (10 mg/ml) on the same membrane. Thereafter, membranes were washed twice in 2x sodium chloride-sodium citrate (SSC; 1x SSC contains 15 mmol sodium citrate/liter and 150 mmol sodium chloride/liter)/0.1% (w/v) sodium dodecylsulphate (SDS) at room temperature, for 15 min each, and once in 0.1x SSC-0.1% SDS at 42°C for 30 min by shaking. Finally, the membranes were exposed to a Fuji medical X-ray film at -85°C using an intensifying screen. The film was subsequently developed using conventional procedures. The membranes were used for rehybridization with GAPDH cDNA probe to control for variation in gel loading, pre-existing [^{32}P]-labelled probe on the membranes was removed by boiling the membranes in 0.1x SSC-0.1% SDS until background counts were less than 5 c.p.s. Hybridization intensities of each specific mRNA were quantified using the Arcus II computer-assisted image system (PDI Inc., Huntington Station, NY, USA). Results are expressed as arbitrary units of $P450_{scc}$ /GAPDH mRNA ratios.

Progesterone RIA

The progesterone concentration in the unextracted medium was measured by RIA as previously described (24). The antiserum to progesterone-11α-BSA (C467-B4) was supplied by Dr. J.E. Hixon (Department of Veterinary Biosciences, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA) and used at the dilution of 1:25,000 in 0.01 M Tris buffer (pH 7.4). The sensitivity of the assay was 12.5 pg per assay tube. The intra-assay variation, determined by duplicates of three dose levels of the control medium from the corpora luteal cell pool, was less than 10%.

Statistical Analysis

All progesterone values were expressed as the mean ± SEM of pooled data from two to four experiments. Two means were compared using Student's *t*-test. Where there were more than two means, significant differences between means were determined by ANOVA. The means were then analyzed by Fisher's probable least-squares difference multiple comparison.

Results

Dose-Dependence Effect of FSH on the Expression of P450scc mRNA and Progesterone Production

To determine the optimal dose of FSH, granulosa cells were cultured for 48 h with various doses of FSH, and P450scc mRNA levels were determined. As shown in Figure 1a, a dose-dependent relationship between P450scc mRNA expression and FSH treatment was readily seen in pig granulosa cells as determined by Northern blot analysis. The P450scc mRNA was low in unstimulated cells but could be seen with levels of FSH greater than 1 ng/ml. The level of P450scc mRNA continued to increase, reaching a maximal value at 200 ng/ml. In all further experiments, a dose of 100 ng/ml was used.

Progesterone production in the culture medium from the same experiment is shown in Figure 1b. FSH at concentrations used caused a dose-dependent increase in progesterone production, reaching a maximal effect at a concentration of 100 ng/ml.

Effect of Treatment with Dexamethasone on the Expression of P450scc mRNA and Progesterone Production

To investigate the effects of dexamethasone on the expression of P450scc mRNA, granulosa cells were incubated for 48 h with or without increasing concentrations of dexamethasone in the presence or absence of FSH. As shown in Figure 2, dexamethasone treatment resulted in a dose-dependent increase in constitutive and FSH-induced expression of P450scc mRNA. Densitometric analysis of the autoradiogram revealed a 19.8-fold increase in P450scc mRNA level in the presence of FSH and a 11.2-fold rise of the FSH effect by concomitant treatment of the cultured cells with 100 nM dexamethasone. Dexamethasone at the dose of 100 nM also increased constitutive expression of P450scc mRNA by about 9-fold over control value.

Results shown in Figure 3 show that dexamethasone at concentrations of 100 nM or greater significantly ($P<0.05$) increased basal and FSH-stimulated progesterone production.

Table 2. Effect Of Dexamethasone (DEX) On 8-Br-cAMP-Stimulated Progesterone Production By Cultured Pig Granulosa Cells[a]

	Progesterone (ng/dish)	
8-Br-cAMP (mM)	- DEX	+ DEX
0	9.50 ± 2.18	26.30 ± 4.82[b]
0.1	332.96 ± 31.83	698.12 ± 64.54[b]
0.5	1876.16 ± 241.14	2718.90 ± 222.70[b]
1.0	1065.30 ± 117.91	3614.40 ± 194.76[b]

[a]Granulosa cells were cultured with 100 nM DEX (+ DEX) or without DEX (- DEX) in the presence or absence of increasing concentrations (0.1 – 1.0 mM) of 8-Br-cAMP. The progesterone concentration was measured in duplicate samples from two culture dishes at each dose of 8-Br-cAMP after 48-h incubations. Values are the mean ± SEM from four independent experiments. Significance of difference between + DEX and – DEX for each dose of 8-Br-cAMP: [b]$P < 0.05$.

In the time-course experiment, granulosa cells were cultured with or without a constant dose of dexamethasone (100 nM) in the absence or presence of FSH (100 ng/ml) for 12, 24, or 48 h (Fig. 4). Treatment with dexamethasone caused significant ($P<0.05$) increases in basal and FSH-stimulated progesterone production at times greater than 24 and 12 h, respectively.

Effect of Treatment with Dexamethasone on Forskolin- and 8-Br-cAMP-Stimulated Expression of P450scc mRNA and Progesterone Production

The influence of dexamethasone upon the ability of forskolin (10 μM), an activator of adenylate cyclase, and 8-Br-cAMP (1 mM), a cAMP analog, to stimulate P450scc mRNA expression was also investigated. Dexamethasone (100 nM) administration produced a 2.4-fold increase in P450scc mRNA level over forskolin-stimulated value (Fig. 5a). Results in Table 1 show that incubation of pig granulosa cells with a constant dose of dexamethasone (100 nM) for 48 h stimulated progesterone production at least by 31% at every dose of forskolin tested.

Similar results were obtained with the use of 8-Br-cAMP as an inducer of P450scc mRNA expression (Fig. 5b). Densitomeric analysis of the autoradiogram shown in Figure 5b revealed a 4.4-fold increase in P450scc mRNA content upon dexamethasone treatment of 8-Br-cAMP-treated cells. Results in Table 2 show that treatment with a constant dose of dexamethasone (100 nM) for 48 h increased progesterone production at least by 20% at every dose of 8-Br-cAMP tested

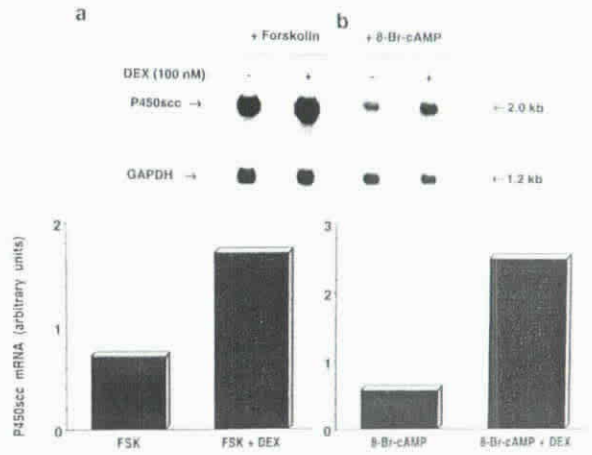

Fig. 5. Effect of treatment with dexamethasone (DEX) on forskolin (FSK)-stimulated (a) and 8-Br-cAMP-stimulated (b) P450scc mRNA expression. Cell culture, incubations, RNA extraction, and analysis were conducted as described in Figure 2 with the exception that the granulosa cells were cultured with (+) or without (-) DEX (100 nM) in the presence of FSK (10 μM) or 8-Br-cAMP (1 mM). The data represent the results from a single experiment that was performed at least three times with similar results.

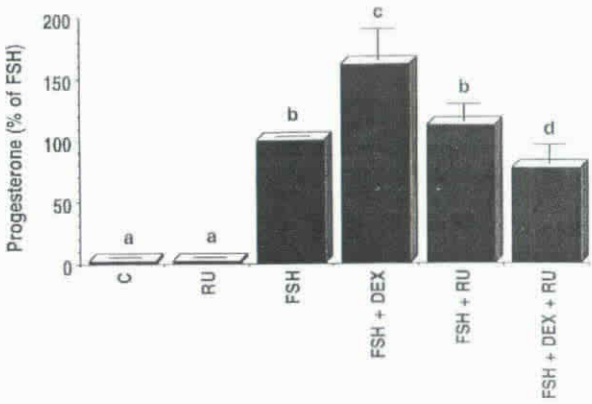

Fig. 6. RU-486 (RU) effects on dexamethasone (DEX) potentiation of FSH-stimulated progesterone production. Pig granulosa cells were treated for 48 h with FSH (100 ng/ml), DEX (100 nM), and RU (1 μM) in combinations shown. The progesterone concentration was measured in duplicate samples from two culture dishes at each treatment. Values are the mean ± SEM from two independent experiments. C, control. Bars with different letters differ significantly ($P < 0.05$).

Effect of RU-486 on Dexamethasone Enhancement of Progesterone Production

The glucocorticoid receptor antagonist, RU-486, was used to determine whether the effects described above were mediated by the glucocorticoid receptor. Granulosa cells were cultured for 48 h with FSH (100 ng/ml) in the presence or absence of dexamethasone (100 nM) and with or without RU-486 (1 μM). The results are

presented in Figure 6. RU-486 antagonized the stimulatory effect of dexamethasone on progesterone production.

Discussion

In this study, we have demonstrated that the glucocorticoid dexamethasone is able to enhance both basal and FSH-induced P450scc mRNA expression and progesterone production in pig granulosa cells. The dexamethasone effect on steroidogenesis is dose- and time-dependent. These results indicate that dexamethasone enhances granulosa cell steroidogenesis by up-regulate P450scc mRNA expression. We also demonstrated that RU-486 that can displace dexamethasone from the glucocorticoid receptor reversed the effect of dexamethasone on progesterone production. This strongly suggests a glucocorticoid receptor-mediated mechanism of enhancement of FSH-induced progesterone production. The RU-486 effect is specific, as this compound by itself does not influence progesterone production induced by FSH nor does it have any effect on the basal level of progesterone.

The studies reported here provide evidence for a local effect of glucocorticoids on the ovary by the direct demonstration of the stimulatory activity of dexamethasone on the production of progesterone in response to FSH. Our present observations on pig granulosa cells confirm the previous findings observed in rat preantral primary granulosa cells, which showed that dexamethasone enhanced the FSH-induced progesterone production (1, 17). In contrast, dexamethasone has been shown to inhibit LH-stimulated pregnenolone production in human granulosa-lutein cells (29). The discrepancy in results may be explained by the differences in experimental conditions used, such as the status of differentiation of the follicles. Granulosa cells from 3- to 5-mm pig follicles or immature diethylstilbestrol-treated rats might be considered nontransformed and undifferentiated or in the process of differentiation, while human granulosa-lutein cells would be more mature and highly differentiated. It has been reported that in rat and human granulosa cells that have not luteinized in vitro, glucocorticoids can potentiate the cAMP and steroid responses to LH and FSH (1, 3, 6, 13). Thus, it would appear that the effects of glucocorticoids on the steroidogenic activity of a given ovarian cell type may enhance FSH action in granulosa cells in the follicular phase of the ovarian cycle but predominantly attenuate LH-stimulated steroidogenesis during the luteal phase. The use of our experimental model, however, suggests that glucocorticoids in vivo may act directly on the ovary to modulate follicular steroidogenesis.

In our studies, the effective stimulatory dose of dexamethasone was at 100 nM (51.6 ng/ml), which is less than circulating levels of cortisol found in pigs after corticotropin administration or application of an acute stressor (20), but lies above the circulating levels under nonstressed conditions. Because greater than 90% of circulating glucocorticoids are bound by transcortin (39, 44), the dose (100 nM) of dexamethasone, a more potent synthetic glucocorticoid, may represent an overestimate of the amount of in vivo sustained stressed conditions, and the effect seen is probably more pharmacological than physiological. Thus, the influence of glucocorticoids in normal follicular steroidogenesis may be minimal. However, under the conditions of our experiment, the demonstration of a direct stimulatory effect of dexamethasone on progesterone production suggests that other mechanisms to diminish ovarian function during stress may exist. The possibility that glucocorticoids may act directly on the target tissues of progesterone remains to be investigated.

The stimulatory action by dexamethasone was prevented in the granulosa cell by RU-486. Specific receptors to glucocorticoids have been identified in granulosa cells (35), and glucocorticoids have been shown to exert direct effects on ovarian steroidogenesis (1, 3). However, RU-486 also blocks progesterone receptors (16). Studies in human granulosa cells indicate that progesterone regulates granulosa cell proliferation and differentiation in an autocrine-paracrine manner (5). Another study of pig granulosa cells with the synthetic progesterone R-5020 showed that the involvement of progesterone in its own production was minimal (33). Therefore, our results indicate that the effects of dexamethasone on progesterone production in pig granulosa cells are mediated through the glucocorticoid receptor. Because little information is available on the synthesis of the glucocorticoid receptor and the process of upregulation of its hormone receptor in the ovary in states of glucocorticoid excess, we do not know if the regulation of the glucocorticoid receptor by dexamethasone exists. Further studies are necessary to define the role that glucocorticoids play in the regulation of the glucocorticoid receptor gene expression in the ovary.

Whether the stimulatory mechanism of glucocorticoids is mediated prior or distal to cAMP formation was examined by investigation of the effect

of dexamethasone on forskolin- and 8-Br-cAMP-stimulated progesterone production. Dexamethasone treatment enhanced forskolin-stimulated progesterone production. This finding suggests that dexamethasone may stimulate adenylate cyclase activity, resulting in the induction of cAMP formation. In addition, to induce cAMP formation, our observation that dexamethasone treatment also potentiated 8-Br-cAMP-stimulated progesterone production indicated that the action of dexamethasone is exerted, at least in part, at a point distal to the generation of cAMP. Further studies involving direct measurement of follicular cAMP production and the activities of adenylate cyclase is needed to elucidate this point.

With regard to the direct cellular mechanisms responsible for the effect of glucocorticoids on hormone-induced steroidogenesis, our present study demonstrates the effect of dexamethasone to stimulate FSH-induced expression of P450scc mRNA in pig granulosa cells in primary culture. This stimulation of P450scc may in part account for the increase in progesterone production. Dexamethasone may enhance FSH-stimulated P450scc mRNA level through either transcriptional regulation or effects on mRNA stability. Dexamethasone has been shown to increase retinoic acid-stimulated gene transcription of the S14 gene in 3T3-F442A preadipocytes (22). Dexamethasone also increases mitochondrial RNA concentrations 4-fold in the rat hepatoma cell line H-4-II-E by increased gene transcription (41). Dexamethasone may also be enhancing FSH-mediated transcription. Studies in bovine adrenocortical cells have shown that dexamethasone will affect reporter gene activity of transfected constructs of bovine P450scc containing cAMP-response sequences (40). Finally, dexamethasone may increase the stability of P450scc mRNA. A glucocorticoid-responsive mRNA-stabilizing element has been described for the liver enzyme phosphoenolpyruvate carboxykinase (31). Our data, which demonstrate that glucocorticoids increase the steady state level of P450scc mRNA, however, cannot distinguish between changes in the rate of P450scc gene transcription and changes in the P450scc mRNA stability. Because "orphan" nuclear hormone receptors have been shown to bind to the regulatory regions of P450scc gene. These receptors include COUP and steroidogenic factor 1 (21). It is not known, however, if glucocorticoids are ligands for such receptors, and consequently, a mediation of the effects of dexamethasone on P450scc gene expression through these receptors cannot be ruled out. Furthermore, the StAR protein has recently been shown to be a factor necessary for cholesterol transport into adrenal and gonadal mitochondria, which is the regulated rate-limiting step in steroidogenesis (37). Whether dexamethasone potentiates FSH-induced synthesis of StAR protein in cultured pig granulosa cells also needs to be examined.

We conclude from the results obtained that in pig granulosa cells that are not luteinized in vitro, dexamethasone acts to potentiate the FSH-stimulated P450scc gene expression and progesterone production. These observations raise the possibility that glucocorticoids in vivo may have a role in maintaining the physiological function of granulosa cells in the follicular phase of the ovarian cycle. Additional studies are necessary to clarify the mechanism(s) involved in the stimulating effect on the expression of P450scc gene.

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