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Prostaglandin F2α Reduces Steroidogenic Acute Regulatory (StAR) Protein Messenger Ribonucleic Acid Expression in the Rat Ovary

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Steroid biosynthesis begins with the enzymatic conversion of cholesterol to pregnenolone. This reaction is catalyzed by the cytochrome P450 side-chain cleavage enzyme (P450scc), which is located on the matrix side of the inner mitochondrial membrane. Although the rate-limiting enzymatic step in steroidogenesis is the conversion of cholesterol to pregnenolone by the side-chain cleavage enzyme, the true rate-limiting step in this process is the delivery of cholesterol to the inner mitochondrial membrane. Steroidogenic acute regulatory (StAR) protein is thought to mediate the rapid increase in steroid hormone biosynthesis in response to tropic hormones by facilitating cholesterol transport to the inner mitochondrial membrane. Cholesterol transport across the inner mitochondrial membrane has also been implicated as the target for prostaglandin F2α's (PGF2α) antisteroidogenic activity. Since cholesterol delivery to the P450scc is a rapidly regulated step in steroidogenesis, StAR mRNA levels were examined after the administration of a luteolytic injection of PGF2α. The results of this investigation revealed that both major StAR RNA transcripts were decreased in the ovary, 10 d after ovulation, following PGF2α administration. Serum progesterone levels were decreased following PGF2α administration in parallel with the decreased expression of StAR. Following PGF2α treatment, ovarian StAR transcripts at 3.4 and 1.6 kb were reduced 4-fold ($p < 0.01$) and 2.5-fold ($p < 0.025$), respectively, after 4 h. Ovarian P450scc mRNA levels were also reduced (70%) 4 h after PGF2α injection. Time course experiments following PGF2α administration showed a significant decrease in StAR expression as early as 30 min ($p < 0.02$) following injection. In contrast to StAR's expression after PGF2α administration, StAR mRNA levels were elevated in response to human chorionic gonadotropin (hCG) 3 h postinjection. Administration of PGF2α followed by hCG injection effectively blocked induction of StAR expression. StAR mRNA levels were reduced 1.5-fold relative to control animals and 3.5-fold relative to the hCG-treated animals ($p < 0.05$). The levels of serum progesterone paralleled the change in ovarian StAR mRNA in all experiments. This study provides the first evidence that StAR mRNA expression is mediated by prostaglandins in the rat ovary further supporting its important role in the regulation of steroid hormone biosynthesis.

Key Words: PGF2α; StAR; cholesterol; steroidogenesis; ovary; progesterone.

Introduction

In the ovary, if pregnancy does not occur, it is essential that the corpus luteum regress, allowing for the initiation of a new reproductive cycle. At the onset of luteal regression, there is a precipitous decline in serum progesterone concentration (Butcher et al., 1974; Diekman et al., 1978; Baird, 1984) followed by a decline in luteal weight (Diekman et al., 1978; Braden et al., 1988). In the pig, cow, sheep, and rat, PGF2α is believed to be the physiological agent responsible for causing corpus luteum regression at the end of a nonfertile cycle (Hansel and Convey, 1983; Knickerbacker et al., 1988; Gadsby et al., 1990) and there is growing evidence that PGF2α is also important for corpus luteum involution in humans (Bennegard et al., 1984; Ichikawa et al., 1990; Abayasekara et al., 1993). In the pregnant and pseudopregnant rat, a high correlation between luteal PGF2α content and the demise of luteal function has been reported, clearly demonstrating a role for luteal PGF2α in rat luteolysis (Olofsson and Selstam, 1988; Olofsson et al., 1990; Cao and Chan, 1993). Corpus luteum regression is characterized by functional and structural alterations that result in the loss of steroid production by the luteal cells (Abayasekara et al., 1993). PGF2α has been shown to depress cAMP accumulation and serum progesterone levels (Lahav et al., 1989), and to antagonize luteinizing hormone (LH)-stimulated steroid production in luteal tissue (Lahav et al., 1989).

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In the rat corpus luteum, PGF2α has been shown to have a very rapid antigonadotropic effect in vitro (Khan et al., 1979). PGF2α addition to cultured rat luteal cells in combination with LH, suppressed LH stimulation of adenylate cyclase and progesterone secretion (Thomas et al., 1978). Furthermore, in the intact rat corpus luteum, Lahav et al. (1976) have demonstrated that PGF2α prevents the normal LH-induced increase in cAMP accumulation. Thus, it is clear that PGF2α has rapid antagonizing effects on LH stimulation of progesterone secretion both in vitro and in vivo.

Following PGF2α-induced luteolysis in sheep and cows, corpora lutea accumulate substantial stores of lipids (Umo, 1975; Heath et al., 1983) implying that the uptake of cholesterol is not impaired. An investigation by Pate and Condon (1989) concluded that following PGF2α treatment, luteal tissue could bind and internalize lipoproteins, but determined that low-density lipoprotein (LDL)-derived cholesterol utilization for steroidogenesis was abrogated. In the rat, PGF2α administration also had no effect on lipoprotein binding to luteal membranes, suggesting that PGF2α treatment does not affect the uptake of lipoproteins (Rajkumar et al., 1988). Grusenmeyer and Pate (1992) have since suggested that PGF2α inhibits progesterone production at a site subsequent to cholesterol transport to the mitochondria, or that it may inhibit cholesterol transfer from the outer to the inner mitochondrial membrane. Consistent with this hypothesis, Behrman and Aten (1991) proposed that hydrogen peroxide produced following PGF2α administration alters hormone-sensitive cholesterol transport to the mitochondria of rat luteal cells.

The study by Grusenmeyer and Pate (1992) implicated the transport of cholesterol across the inner mitochondrial membrane as well as subsequent steps in the steroidogenic pathway as possible targets for PGF2α's antisteroidogenic activity. The first enzymatic step following cholesterol transport across the inner mitochondrial membrane is the conversion of cholesterol to pregnenolone, which is catalyzed by P450scc.

The rapid increase in steroid hormone biosynthesis in steroidogenic cells in response to acute hormone stimulation is well documented (Mendelson et al., 1975), and the rate-limiting step of this rapid response appears to be the delivery of the cholesterol substrate to the mitochondrial cytochrome P450 side-chain cleavage enzyme (P450scc) located on the inner mitochondrial membrane (Crivello and Jefcoate, 1980). The major barrier to be overcome in cholesterol delivery to the side-chain cleavage enzyme is the aqueous space between the outer and inner mitochondrial membranes through which this relatively hydrophobic compound must pass. Recently, Clark et al. (1994) isolated and cloned the cDNA for a 30 kDa, LH-induced mitochondrial protein from mouse MA-10 Leydig tumor cells, and have referred to this protein as the StAR protein. This novel protein, which has since been identified in the rat (Sandhoff and McLean, 1996), cow (Hartung et al., 1995), sheep (Juengel et al., 1995), and human (Sugawara et al., 1995), appears to be required for the acute regulation of hormone-induced steroidogenesis (Clark et al., 1994; Sugawara et al., 1995; Clark and Stocco, 1995; King et al., 1995). Since several investigators (Umo, 1975; Heath et al., 1983; Pate and Condon, 1989; Behrman and Aten, 1991; Grusenmeyer and Pate, 1992; Niswender et al., 1994) have suggested that cholesterol transport to the mitochondria is the principal site of PGF2α's antisteroidogenic activity; it is logical to consider whether StAR may provide a locus for the inhibitory actions of this prostaglandin in the negative regulation of ovarian steroidogenesis.

While PGF2α appears to have multiple biological actions, all of which have a negative effect on luteal function, this investigation focused on the actions of this prostaglandin on the expression of StAR and the possibility that a reduction in StAR mRNA levels may directly correlate with PGF2α's antisteroidogenic action on cholesterol transport capacity.

Results

In order to examine the normal ovarian steady-state StAR mRNA levels during the reproductive cycle, rats were treated with pregnant mare's serum gonadotropin (PMSG) to induce follicular development. Rats ovulate approx 72 h following PMSG treatment. The results of this experiment (Figs. 1 and 2) indicate that immature rat ovaries express very little StAR mRNA. Following PMSG injection, StAR mRNA levels increased greatly. At 72 h, post-PMSG injection StAR mRNA levels had increased 20-fold relative to the control animals. The 3.4 kb transcript was increased another seven-fold by day 4 postovulation before StAR levels reached a plateau that was maintained throughout the luteal phase. Ovarian P450scc mRNA levels paralleled StAR levels in this experiment, but reached maximal levels of expression on day 6 postovulation. Serum progesterone levels increased in parallel with StAR expression up to day 15 postovulation (Table 1). On day 20, however, serum progesterone levels declined to control values whereas StAR expression remained somewhat elevated (Fig. 2).

To determine whether PGF2α could exert its antisteroidogenic effect directly on StAR mRNA expression, ovarian tissue was examined on day 10 of pseudopregnancy before (t_0), and 4 h after PGF2α injection (250 μg). The results of this experiment indicate that ovarian StAR mRNA expression was down regulated (75%) by PGF2α 4 h following injection ($p < 0.01$, $p < 0.025$ for the 3.4 and 1.6 kb transcripts, respectively) (Fig. 3). P450scc mRNA expression was also reduced (70%) 4 h after PGF2α injection. The decline in StAR mRNA expression paralleled a significant decrease (50%; $p < 0.001$) in serum progesterone levels (control = 127.0 + 6.7 ng/mL; 4 h post-PGF2α = 62.9 ± 12.7 ng/mL) 4 h after PGF2α injection.

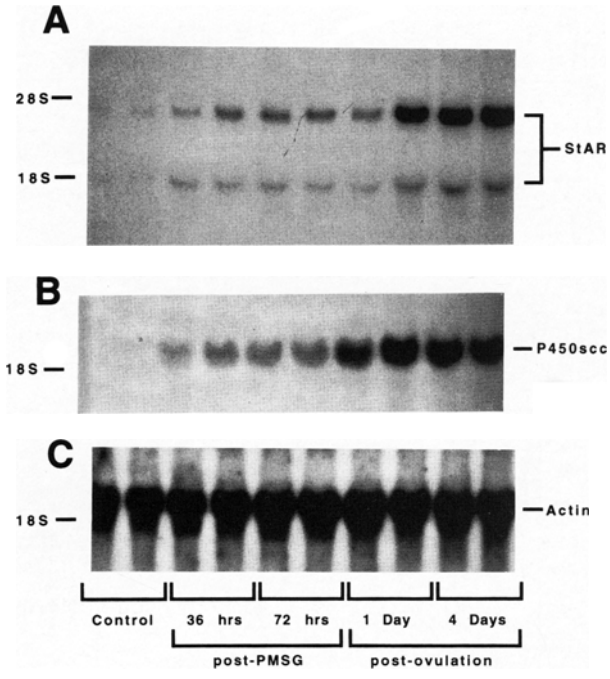

Fig. 1. Regulation of StAR mRNA expression in rat ovaries by PMSG. Twenty-eight day old Sprague-Dawley rats were injected with 8 IU PMSG to induce follicular development. Ovaries were obtained prior to PMSG injection (control ovaries) and at 36 and 72 h following PMSG administration. Ovaries were also obtained 1 and 4 d postovulation. Northern blot analysis of StAR mRNA levels in the ovary following PMSG treatment indicates an increase in StAR mRNA during follicular and luteal development. This blot was probed sequentially for StAR **(A)**, P450scc **(B)**, and β-actin **(C)**.

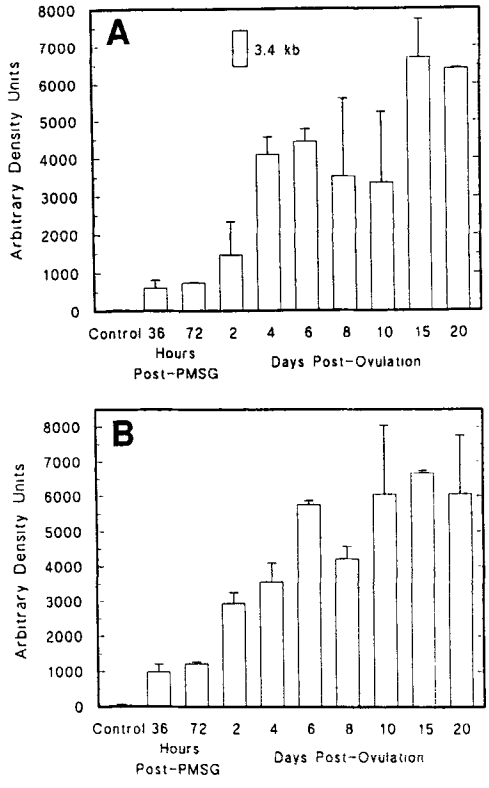

Fig. 2. Densitometric analysis of StAR and P450scc mRNA levels in the ovary following PMSG-induced follicular and luteal development. Autoradiographs were scanned with a densitometer and the results presented. **(A)** The 3.4 kb StAR transcript was significantly increased in the ovary following PMSG injection. **(B)** P450scc mRNA levels were also increased following PMSG administration. Both StAR and SCC mRNA levels remained elevated through day 20 compared to control animals (non-PMSG treated animals).

To determine the time course of reduction in StAR mRNA levels following PGF2α administration, ovarian tissue was examined on day 10 of pseudopregnancy before (t_0) and at 30 min, 1, 2, 3, and 4 h post-PGF2α injection. The results of this experiment (Fig. 4) indicate that StAR mRNA levels begin to decline as early as 30 min ($p < 0.02$) following PGF2α administration and continue to fall up to 4 h postinjection. Serum progesterone levels were also declining by 30 min post-PGF2α injection. By 4 h after PGF2α injection, serum progesterone was markedly reduced relative to control animals as noted in the previous experiment.

To determine what effect a gonadotropin would have on StAR mRNA levels after PGF2α injection, ovarian tissue was examined on day 10 of pseudopregnancy before any injections and 4 h after injection with PGF2α and hCG. The results of this experiment indicate that ovarian StAR mRNA induction by hCG was blocked by PGF2α (Fig. 5). Overall, StAR mRNA levels were increased by hCG and decreased by PGF2α. The effect of both hCG and PGF2α on StAR mRNA levels indicated a small reduction relative to control animals (1.5-fold), but a major reduction (3.5-fold) relative to hCG-treated animals (Figs. 5 and 6A). The 3.4 kb transcript was reduced to 75% of control levels ($p < 0.05$). The change in serum progesterone levels directly paralleled the expression of StAR mRNA in this experiment (Fig. 6B).

Serum progesterone levels were increased in response to hCG treatment (4 h post-hCG). This increase in serum progesterone was blocked by pretreatment with PGF2α (4 h post-PGF2α/hCG) (Fig. 6B).

Discussion

This study is the first to demonstrate that rat ovarian StAR mRNA expression is reduced following PGF2α treatment. The decline in StAR mRNA levels following PGF2α injection is consistent with this prostaglandin's suggested luteolytic function to alter cholesterol transport to the mitochondrial P450scc enzyme. Furthermore, when PGF2α was administered prior to hCG injection, StAR expression in the ovary was significantly reduced. These results suggest that PGF2α may reduce intracellular cholesterol transport by suppressing the expression of basal and gonadotropin-stimulated steroidogenesis.

The results of this study indicate that both StAR and P450scc are induced in the immature ovary by PMSG and that while StAR message is increased in response to hCG, P450scc mRNA levels remain elevated independent of

Table 1
Serum Progesterone Levels During Follicular and Luteal Development in the Super-Ovulated Rat

	Hours post-PMSG treatment			Days postovulation				
	Control	36 h	72 h	1 d	8 d	10 d	15 d	20 d
Progesterone (ng/ml)	4.0 ± 1.0	15.5 ± 3.5	26.0 ± 5.0	53.0 ± 6.0	73.0 ± 55.0	217.5 ± 13.5	118.5 ± 75.0	26.0 ± 2.5

Mean Progesterone levels ± SEM.

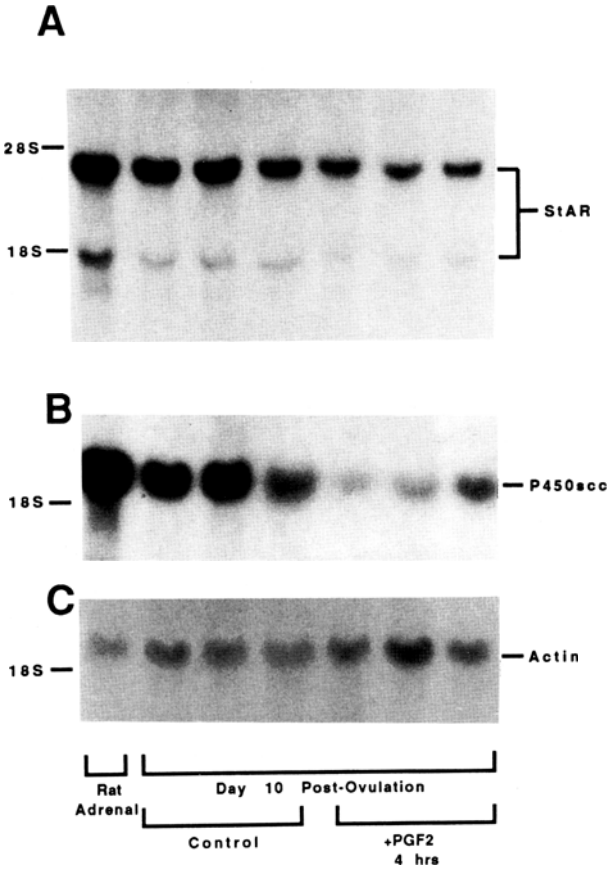

Fig. 3. Regulation of StAR mRNA expression in rat ovaries by PGF2α. Twenty-eight day old Sprague-Dawley rats were injected with 8 IU PMSG and 10 d after ovulation were injected with PGF2α (250 μg). Ovaries were obtained prior to PGF2α administration (control; t0) and at 4 h post-PGF2α injection. Northern blot analysis of StAR mRNA in the ovary following PGF2α treatment. This blot was probed sequentially for StAR **(A)**, P450scc **(B)**, and β-actin **(C)**.

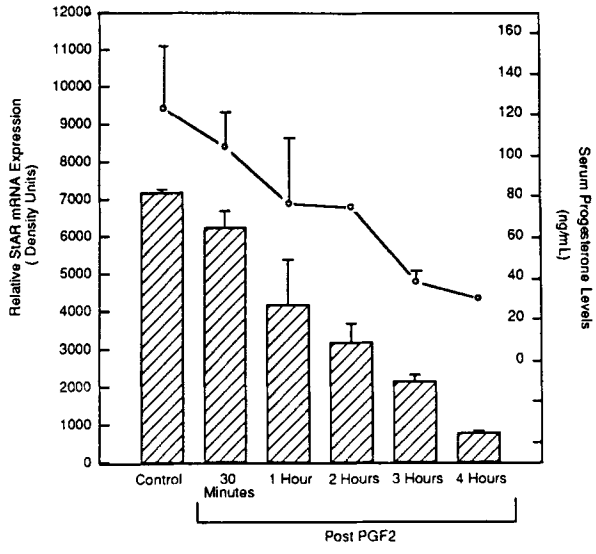

Fig. 4. Time course of reduction of StAR mRNA levels in rat ovaries by PGF2a. Ten days following ovulation, rats were injected with PGF2α (250 μg). Ovaries were obtained prior to PGF2α administration (control; t0) and at 30 min, 1, 2, 3, and 4 h post-PGF2α injection. Northern blot analysis of StAR mRNA in the ovary following PGF2α treatment. Autoradiographs were scanned with a densitometer and the results presented. Serum progesterone levels were assayed and the results presented.

hCG administration following corpus luteum development. P450scc mRNA has been shown to be rapidly (within 7 h) and maximally increased during hCG-induced luteinization (Goldring et al., 1987). This study also suggested that the maintenance of P450scc mRNA and P450scc protein in rat corpora lutea was due to the constitutive expression of P450scc mRNA. Whereas the study by Sandhoff and McLean (1996) confirmed that P450scc mRNA is constitutively expressed in rat corpora lutea, it also demonstrated that in response to acute hormone stimulation with hCG, P450scc mRNA levels remained unchanged during the rapid increase in steroid production. This rapid increase in steroid production directly paralleled the increase in StAR mRNA expression. In the current investigation, PGF2α administration, in addition to reducing StAR expression, reduced the level of P450scc mRNA in the ovary within 4 h of treatment. This demonstrates that PGF2α's antisteroidogenic activity is exerted at multiple sites within ovarian cells to reduce progesterone production. Based upon our previous findings (McLean et al., 1995) we know that the antisteroidogenic action of PGF2α on P450scc appears to be transient and that P450scc mRNA levels rebound within 8 to 12 h.

Luteolytic action of PGF2α in the rat was originally suggested to be a result of an increase in 20α-hydroxysteroid dehydrogenase (Pharriss and Wyngarden, 1969) that converts progesterone to the inactive derivative 20α-dehydroprogesterone (20α-DHP). However, studies have demonstrated that the decline in serum progesterone is rapid following PGF2α treatment (within 30 min) (Khan and Rosberg, 1979; Lahav et al., 1989), whereas an increase in 20α-DHP was not noted until 9 h following

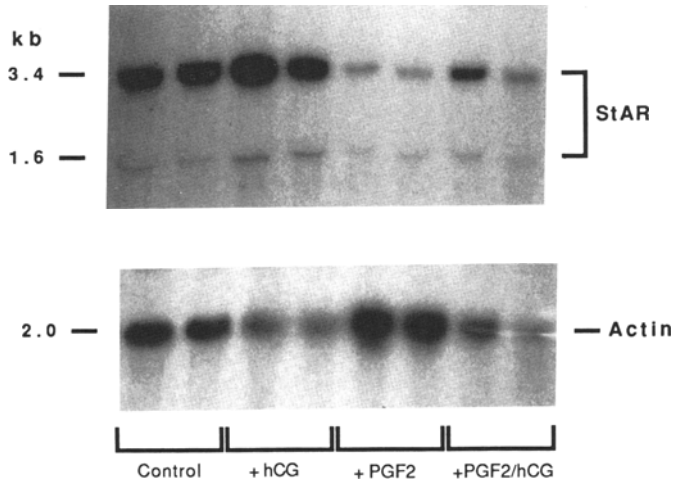

Fig. 5. Regulation of StAR mRNA expression in rat ovaries by hCG and PGF2α. Twenty-eight day old rats were injected with 8 IU PMSG and 10 d after ovulation were given a luteolytic injection of PGF2α (250 μg) followed by injection of hCG (50U) 30 min later. Ovaries were obtained prior to administration of hormones (t0) and 4 h after the PGF2α/hCG injection protocol. Northern blot analysis of StAR mRNA in the ovary following PGF2α/hCG injection. This blot was probed sequentially for StAR and β-actin.

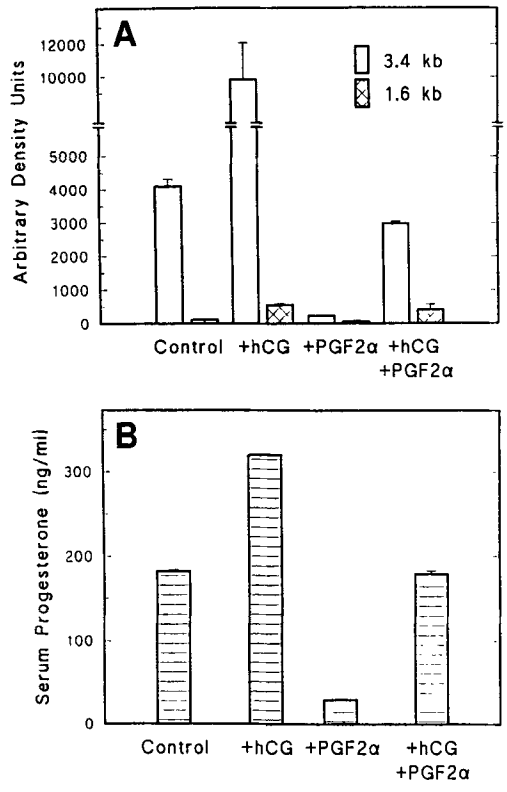

Fig. 6. Densitometric analysis of StAR mRNA levels **(A)** and serum progesterone levels **(B)** in the ovary following PGF2α/hCG treatment. Autoradiographs (Fig. 5) were scanned with a densitometer and the results presented. (B) Serum progesterone levels in the same animals were assayed and the results presented.

PGF2α injection. Bussman (1989) suggests that the decrease in intraluteal progesterone may actually signal 20α-hydroxysteroid dehydrogenase induction. Thus, the initial luteolytic action of PGF2α is to inhibit steroidogenesis rather than induce the inactive progestin.

Early studies demonstrated that steroid production in response to hormone stimulation had an absolute requirement for the synthesis of new proteins (Garren et al., 1965; Krueger and Orme-Johnson, 1983). As a result of these investigations, it was hypothesized that the acute production of steroids was dependent on a highly labile (Pon and Orme-Johnson, 1986), cycloheximide-sensitive protein (Krueger and Orme-Johnson, 1988) that appeared in response to hormone treatment (Pon and Orme-Johnson, 1986; Epstein and Orme-Johnson, 1991) and functioned to transfer cholesterol to the inner mitochondrial membrane (Pon and Orme-Johnson, 1988). Since the true rate-limiting step in steroid production appears to be the delivery of cholesterol to the side-chain cleavage enzyme (Crivello and Jefcoate, 1980), protein mediators of this transfer have been the subject of intense investigation (reviewed in Stocco and Clark, 1996). Acute regulation of cholesterol transport in response to tropic hormones is well documented (Garren et al., 1965; Mendelson et al., 1975; Cooke et al., 1975) and the studies by several laboratories (Clark et al., 1994; Lin et al., 1995; Sugawara et al., 1995) support the concept that StAR enhances steroid production.

Several studies (Khan and Rosberg, 1979; Pate and Condon, 1984; Pate and Condon, 1989; Lahav et al., 1989) have shown that PGF2α suppresses LH-stimulated (Jordan, 1981; Pang and Behrman, 1981; Benhaim et al., 1987; Pate and Condon, 1989) and lipoprotein-stimulated (Pate and Nephew, 1988; Pate and Condon, 1989; Grusenmeyer and Pate, 1992) progesterone production. Whereas the decline in progesterone production is a consistent finding following prostaglandin treatment, the decrease in steroid production was not the result of a reduction in LDL or high-density lipoprotein (HDL) uptake in bovine (Pate and Condon, 1989; Grusenmeyer and Pate, 1992) luteal cells. In the rat, PGF2α does not alter HDL (Rajkumar et al., 1985) or LDL binding (Rajkumar et al., 1988) in the corpus luteum. Since PGF2α does not appear to alter the uptake of lipoprotein-carried cholesterol into ovarian cells, its anti-steroidogenic action must impede the subsequent use of the lipoprotein-derived cholesterol for steroidogenesis. Unique to our investigation was the discovery that PGF2α can reduce the expression of StAR and P450scc mRNA. The decline in StAR and P450scc message directly paralleled the decline in serum progesterone in these studies. The decline in StAR expression would be consistent with the suggestions of several investigators (Umo, 1975; Heath et al., 1983; Pate and Condon, 1989; Behrman and Aten, 1991; Grusenmeyer and Pate, 1992;

Niswender et al., 1994) who postulated that PGF2α alters cholesterol transport to or within the mitochondria. Consistent with this notion, our laboratory has recently shown that the expression of the putative intracellular cholesterol transport protein, sterol carrier protein-2 (SCP2), is reduced following PGF2α administration (McLean et al., 1995). These studies, in conjunction with the results presented here, suggest that PGF2α may alter cholesterol transport to the mitochondria as well as cholesterol movement across the mitochondrial membrane. Thus, both SCP2 and StAR, which comprise an intracellular cholesterol transport system, may be disrupted by PGF2α. The mechanism(s) through which this prostaglandin acts to alter SCP2 and StAR expression remains to be clarified. This study is the first to demonstrate that PGF2α is able to block hCG-induced steroid production at the level of StAR expression. This significant action of PGF2α to prevent the hCG/cAMP-mediated increase in StAR expression further supports StAR's critical role in regulating steroid production.

StAR appears to require posttranslational modification to function, and alterations in the phosphorylation state of this protein, although not addressed in this study, may also be of great physiological relevance to the regulation of cholesterol transport. Evidence is accumulating that the antisteroidogenic effects of PGF2α are mediated through the activation of the PKC second messenger system, whereas the luteolytic actions of PGF2α are most likely a result of the process of apoptosis (Rodway et al., 1991; Wiltbank et al., 1991). Protein kinase C (PKC) has been implicated in the control of a number of cellular processes, including differentiation, gene expression, and hormone release. In rat luteal cell suspensions incubated with the PKC agonist, 4β-phorbol myristate 13-acetate (4β-PMA), progesterone accumulation and cAMP levels were shown to be inhibited (Lahav et al., 1989). This activation of PKC by 4β-PMA has been shown to mimic the effects of PGF2α in the rat corpus luteum (Baum and Rosberg, 1987). Pharmacological activation of PKC has been shown to reduce progesterone production from ovine (Wiltbank et al., 1991; Conley and Ford, 1989) and rat luteal cells (Baum and Rosberg, 1987). The site of PKC's post-cAMP-mediated action, and the relevance of this action to StAR expression and function has not been defined and remains to be investigated.

The decline in StAR and P450scc mRNA expression following PGF2α and PGF2α's ability to block the hCG-mediated increase in StAR mRNA as noted in this investigation, support the possibility that PGF2α, as part of its antisteroidogenic action, suppresses StAR and P450scc mRNA expression. This action may contribute to a reduction in intracellular cholesterol transport capacity as well as a reduction in the utilization of cholesterol by P450scc, thus leading to a rapid decline in steroid production.

Materials and Methods

Chemicals, and cDNA probes

PGF2α and hCG were purchased from Sigma Chemical Co. (St. Louis, MO). PMSG was purchased from Diosynth (Chicago, IL). 1,2,6,7-^{3}H(N)-progesterone (104.1 Ci/mmol) was purchased from DuPont-New England Nuclear (Wilmington, DE). [α^{32}P] deoxy-CTP (3000 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL). The progesterone ELISA kit was obtained from Boehringer Mannheim (Indianapolis, IN). BioMax and XAR-5 films were purchased from Eastman Kodak (Rochester, NY). SeaKem and SeaPlaque agarose were purchased from the FMC Corporation (Rockland, ME), and nylon membrane was obtained from Schleicher and Schuell (Keene, NH). TRI-Reagent, Background Quencher, Formazol, Microcarrier Gel-TR, and High Efficiency Hybridization solution were obtained from Molecular Research Center (Cincinnati, OH). The nick translation DNA labeling kit and all restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN). All other chemicals were reagent grade and were obtained from Fisher Scientific (Norcross, GA) or Sigma Chemical Co.

Animals

Twenty-eight-day-old Sprague-Dawley rats were purchased from Harlan Industries of Madison, WI. All procedures for hormone and prostaglandin treatment and the methods for tissue and blood sampling were approved by the University of South Florida Animal Care Committee. Throughout the experiment, animals had free access to food and water and were housed under a 12-h dark, 12-h light cycle. Follicular development and ovulation were induced in rats by injection of 8 IU PMSG (im). Rats ovulate approx 72 h following treatment with 8 IU PMSG (McLean et al., 1995). In one experiment, ovaries were obtained prior to PMSG administration (t0, control) and at 36 and 72 h postinjection, as well as 1, 4, 6, 8, 10, 15, and 20 d postovulation. In a second experiment, rats were treated with PMSG as indicated previously, followed by a single injection of PGF2α (250 μg) on day 10. Ovaries were removed prior to PGF2α injection (t0) and at 30 min, 1, 2, 3, and 4 h post-PGF2α treatment. In a third experiment, rats were treated on day 10 with a single injection of PGF2α (250 μg) followed by injection of hCG (50 units; iv) 30 min later. Ovaries were obtained prior to PGF2α injection (t0) and at 4 h post-PGF2α injection. Tissue was immediately frozen in liquid nitrogen. Serum samples were obtained by cardiac puncture at the time of tissue removal, and serum was stored at –20°C until progesterone was assayed. Rats were euthanized by clipping the diaphragm while under ether anesthesia. In all experiments, four animals were utilized per treatment or time-point.

Serum Progesterone Assay

Progesterone was measured by RIA using 1,2,6,7-^{3}H(N)-progesterone. This assay followed the methods pre-

viously described (McLean et al., 1989) and used the progesterone antibody GDN 337 that was kindly provided by Dr. G.D. Niswender (Colorado State University, Fort Collins, CO). The specificity, validity, and reliability of this RIA have been previously reported (Gibori et al., 1977). Progesterone was also measured by ELISA using the progesterone kit with the ES300 Automated ELISA Reader (Boehringer Mannheim).

RNA Isolation and Electrophoresis

RNA was prepared from ovaries using a modification of the Chomczynski and Sacchi method (Chomczynski and Sacchi, 1987) (TRI-Reagent Method, Molecular Research Center). This method consistently yields 5–8 μg RNA/mg tissue. Tissue (<200 mg) was homogenized in 3 mL of TRI-Reagent with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY), and centrifuged at 12,000*g* for 15 min at 4°C. RNA was precipitated from the aqueous phase with isopropanol, and the RNA pellet was washed in 75% ethanol and resuspended in Formazol (Molecular Research Center, Inc. Cincinnati, OH). RNA was quantified by absorbance at 260 nm in a Beckman DU-70 spectrophotometer (Palo Alto, CA).

For Northern blot analysis, total RNA (20 μg) was denatured at 65°C (15 min) and loaded onto 1% agarose gels containing 3% formaldehyde. Following size fractionation, RNA was blotted onto a nylon membrane (0.45 μm pore size) by capillary transfer, and RNA was fixed to the membrane by UV cross-linking (0.3J/cm^2). Ethidium bromide staining of the gel confirmed that the ribosomal RNAs (18S and 28S subunits) were intact and determined whether equal amounts of RNA were loaded in each lane.

Rat StAR and P450scc cDNA Probes

The isolation and characterization of the rat StAR complementary DNA (cDNA) probe was previously reported (Sandhoff and McLean, 1996). The rat StAR cDNA was shown to hybridize to specific ovarian mRNA transcripts in our previous study. The cDNA probe for rat P450scc was characterized by Goldring et al., (1987) and was obtained from Dr. Joanne Richards (Baylor College of Medicine, Houston, TX).

Northern Blot Analysis

Northern blot hybridizations were performed using a 867 bp rat StAR cDNA, a 1.2 kb rat P450scc cDNA (Goldring et al., 1987), or with a 2.0 kb chicken β-actin cDNA (Sandhoff and McLean, 1996). The cDNA inserts were labeled with [α^{32}P] deoxy-CTP using the nick translation DNA labeling method (Rigby et al., 1977). Northern blots were prehybridized at 62°C for at least 3 h in a 1*M* NaCl, 1% SDS solution containing Background Quencher (Molecular Research Center, Cincinnati, OH). Hybridization was completed in a High Efficiency Hybridization Solution (Molecular Research Center, Cincinnati, OH) containing the ^{32}P-labeled probe (1×10^6 cpm/mL; specific activity = 2×10^8 cpm/μg DNA) at 62°C for at least 16 h. Blots were washed three times at RT (5 min) in 1X SSC/1% SDS and three times at RT (10 min) in 0.1X SSC/0.1% SDS. RNA:cDNA hybrids were visualized on BioMax film using two intensifying screens and a 12–48 h exposure period. Blots were stripped and reprobed with rat P450scc and actin cDNAs. Densitometric analysis was performed on the 2.0 kb β-actin transcript for the standardization of RNA loading.

Data Analysis

The Northern blot results were quantitatively analyzed using a Hoefer Scanning Densitometer (Hoefer Instruments, San Francisco, CA). Minor variations in RNA loading were corrected for using the β-actin cDNA. Serum progesterone was expressed as the mean +/- SEM. Data from these individual parameters were compared by analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test when applicable (Zar, 1974). All analysis was completed using the Statview program with graphics (Abacus Concepts, Berkeley, CA) on a Macintosh IIci computer. A $p < 0.05$ was considered significant for all tests.

Acknowledgments

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