ABSTRACT

The present studies were conducted to address cellular mechanisms responsible for regulating steroidogenic acute regulatory protein (StAR) expression and progesterone synthesis at maturation stages corresponding to both the time of hen follicle selection, as well as before and after the LH surge in preovulatory follicle granulosa cells. A recently published report has established that mitogen-activated protein (MAP) kinase signaling induced by transforming growth factor α (TGFα) treatment blocks FSH-induced differentiation and StAR expression in cultured hen granulosa cells, whereas inhibitors of MAP kinase signaling enhance FSH-induced differentiation. The present in vitro studies demonstrate that in addition to MAP kinase signaling, activation of protein kinase C (PKC) blocks both FSH-induced StAR expression and the initiation of progesterone production in prehierarchical follicle granulosa cells, whereas the pharmacologic inhibitor of PKC, GF109203X, potentiates FSH-induced StAR expression and, as a consequence, the initiation of progesterone synthesis. Moreover, we demonstrate in granulosa cells collected from preovulatory follicles that although an acute increase in progesterone production in response to LH treatment requires rapid transcription and translation of StAR, the magnitude of progesterone production is rate-limited by one or more factors other than StAR (e.g., the P450 cholesterol side-chain enzyme). Finally, the rapid turnover of StAR protein, such as occurs following the withdrawal of LH, provides an additional mechanism for the tight regulation of progesterone production that occurs during the hen ovulatory cycle, and explains the rapid loss of steroidogenesis in the postovulatory follicle. In summary, data reported herein support the proposal that paracrine/autocrine factors (including but not necessarily limited to TGFα) prevent premature expression of StAR in prehierarchical follicle granulosa cells by more than one receptor-mediated signaling pathway. Furthermore, subsequent to follicle selection into the preovulatory hierarchy, StAR transcription and translation is necessary but not sufficient for the full potentiation of the preovulatory surge of serum progesterone.

INTRODUCTION

The appropriate expression of steroidogenic acute regulatory protein (StAR) represents an indispensable component of steroidogenesis within preovulatory follicles and the corpus luteum of the mammalian ovary [1–4]. This protein serves to facilitate transfer of cholesterol to the inner mitochondrial membrane where it undergoes conversion to pregnenolone by the P450 cholesterol side-chain enzyme (P450sec, derived from the CYP11A1 gene). Considering recent evidence for the conservation of StAR and its tissue-specific expression in avian, fish, and amphibian species, one would predict the regulation and function of StAR to be highly conserved within the vertebrate ovary in general [5]. While it is well accepted that ovarian StAR expression in mammals is promoted by FSH and LH predominantly via protein kinase A (PKA) signaling [2], additional factors such as insulin-like growth factor I (IGF-I; [6]), estradiol [7], growth differentiation factor 9 [8], and arachidonic acid [9] may mediate the actions of gonadotropins, or potentiate them, or both. Agonist-induced stimulation of steroid biosynthesis is dependent on active transcription and translation of StAR, and recent efforts have been directed toward identifying the mechanisms by which trophic hormones promote StAR gene transcription [4]. On the other hand, it has been known for some time that activation of protein kinase C (PKC) signaling attenuates gonadotropin-induced steroidogenesis (e.g., [10]), and it is now recognized that this effect can at least in part be explained by PKC inhibition of StAR expression [11].

The cloning and characterization of the chicken StAR cDNA [5] has enabled us to document the induction of StAR mRNA and protein expression as a critical component of FSH-mediated differentiation in hen granulosa cells derived from immature, prehierarchical follicles [12]. This up-regulation of StAR is a prerequisite for the induction of granulosa cell progesterone production, and is associated with the selection of a follicle into the preovulatory hierarchy. Moreover, the full potentiation of FSH-induced StAR expression and progesterone production at this stage of development appears to be dependent on the inhibition of paracrine or autocrine factor-mediated mitogen-activated protein (MAP) kinase signaling (e.g., via transforming growth factor α). Once a follicle reaches the preovulatory stage of development, granulosa cells become LH receptor-dominant [13], and LH-mediated progesterone production occurs in association with the rapid induction of StAR mRNA (within 1 h) and protein (within 3 h) expression [12]. It is well established that a fully potentiated preovulatory surge of serum progesterone is obligatory for ovulation of the largest preovulatory follicle [14, 15].

Accordingly, the present studies were conducted to extend our understanding of mechanisms regulating STAR expression and function in hen granulosa cells both at approximately the time of follicle selection, as well as during the period leading up to and following an LH-induced progesterone surge in preovulatory follicles. A primary difference between these two stages of follicle development rests...
in the fact that undifferentiated cells from prehierarchal follicles fail to express StAR to any significant extent (despite FSH receptor expression), whereas differentiated granulosa cells from preovulatory follicles readily up-regulate the expression of StAR and produce large amounts of progesterone in response to gonadotropin (largely LH) treatment. Moreover, the working hypothesis tested herein is that the full potentiation of FSH-mediated differentiation in FSH receptor-dominant granulosa cells from prehierarchal follicles requires a concomitant removal of tonic inhibitory paracrine signaling. Specifically, experiments were designed to address 1) the role of PKC signaling in modulating FSH-induced differentiation in granulosa cells from prehierarchal follicles, and 2) the relationship between LH-induced StAR expression and the preovulatory progesterone surge in granulosa cells from hierarchal follicles.

MATERIALS AND METHODS

Animals and Reagents

Single-comb white Leghorn hens (Creighton Brothers, Warsaw, IN), 25–35 wk of age and laying regular sequences of more than six eggs, were used in all studies described. Birds were housed individually in laying batteries, provided with free access to feed (Purina Layena Mash; Purina Mills, St. Louis, MO) and water, and were exposed to a 15L:9D photoperiod, with lights-on at midnight. Ovulatory cycles were monitored by recording oviposition on a daily basis. Hens were killed approximately 16–18 h before a midsequence ovulation by cervical dislocation. All procedures described herein were reviewed and approved by the University of Notre Dame Institutional Animal Care and Use Committee, and were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

Recombinant human transforming growth factor α (TGFα) was obtained from PeproTech, Inc (Rocky Hill, NJ), whereas ovine LH (AFP-551B) and recombinant human FSH (lot AFP-8468A) were provided by Dr. A.F. Parlow through the National Hormone and Peptide Program. The MAP kinase inhibitor, U0126, and PKC inhibitor, GF109203X, were from Dr. A.F. Parlow through the National Hormone and Peptide Program. The MAP kinase inhibitor, U0126, and PKC inhibitor, GF109203X, were from Sigma Chemical Co. (St. Louis, MO).

Tissue Collection and Granulosa Cell Cultures

Granulosa layers from the three largest preovulatory (F1, F2, and F3) follicles and undifferentiated prehierarchal (6–8 mm) follicles were collected, combined within their respective group, and dispersed in 0.3% collagenase (type 2; Worthington, Freehold, NJ) before treatment. An aliquot of cells from each group was frozen immediately at −70°C (T0 controls), whereas the remaining cells were plated at 40°C in an atmosphere containing 5% CO₂:95% O₂ in six-well polystyrene culture plates (Falcon 3046; Fisher Scientific, Pittsburgh, PA) at a density of approximately 10⁶/well in 1 ml of M199-Hepes supplemented with Hanks salts (Gibco-BRL, Gaithersburg, MD) plus 1 ml of Dulbecco modified Eagle medium (DMEM) containing 5% fetal bovine serum (FBS; Gibco-BRL). In some instances, granulosa cells from the F1 follicle, or from the combined F2 plus F3 follicle layers, or both, were studied separately because it had previously been established that levels of StAR mRNA were dramatically higher in F1 follicles than in F2 or F3 follicles [5]. Morphologically normal and atretic prehierarchal follicles (3–8 mm) were collected and frozen without separating the granulosa and theca layers, as previously described [16].

In the first set of experiments, levels of StAR mRNA were compared in normal versus atretic prehierarchal follicles. In addition, we evaluated the involvement of MAP kinase signaling versus PKC signaling for initiation of StAR expression and progesterone production in functionally undifferentiated prehierarchal follicle granulosa cells. Granulosa cells were precultured for 1 h in the absence or presence of U0126 (50 μM), then cultured an additional 20 h with FSH (100 ng/ml), or TGFα (50 ng/ml) plus FSH. Doses used were based on previous experiments in hen granulosa cells [12, 17]. Following culture, media and plated cells were collected, cells were pelleted by centrifugation at 500 × g for 5 min, then media and cells were frozen separately at −70°C until media were assayed for progesterone and cells were prepared for total cellular mRNA or protein.

To explore alternative signaling pathways by which TGFα can affect StAR expression, prehierarchal follicle granulosa cells were cultured in the absence or presence of FSH, PMA [18] or FSH plus 162 nm PMA.
for 20 h. In addition, cells were precultured for 1 h in the absence or presence of GF109203X (10 μM) before exposure to PMA or TGFα plus FSH. The dose and preculture time for GF109203X was derived from previous studies of this pharmacologic inhibitor in granulosa/luteal cells [19, 20], and importantly, neither U0126 nor GF109203X induced cell death in hen granulosa cells after 24 h of culture ([12] and unpublished data). Media and cells were collected and processed as described above. In a related experiment, the effectiveness of each inhibitor to block MAP kinase signaling through extracellular signal-regulated kinases 1 and 2 (Erk1/2) was tested by pretreating freshly cultured granulosa cells for an additional 20 min with or without PMA or TGFα. Samples were processed for levels of phosphorylated Erk (Erk-P). Treatment with this dose of TGFα has previously been demonstrated to be an effective inducer of Erk-P in hen granulosa cells [17].

In a second set of experiments, granulosa cells from F2 plus F3 follicles were precultured for 1 h in the absence or presence of actinomycin D (1 μg/ml) or cycloheximide (0.5 μg/ml), then cultured an additional 3 h without or with LH (100 ng/ml) to evaluate the requirement of StAR transcription, translation for progesterone production, or both. In addition, equal numbers of F1 or F2 plus F3 granulosa cells were incubated in the absence or presence of LH for 30 to 180 min to correlate levels of induced StAR protein with progesterone production according to stage within the hierarchy. For both of these experiments, media and cells were collected separately and frozen at −70°C until assayed for progesterone or prepared for total cellular RNA, protein levels, or both. For comparison, levels of P450scc mRNA in freshly collected granulosa cells from the F1 follicle were compared with those in F2 follicles.

To evaluate the turnover of StAR protein in cultured cells, granulosa cells from F1 follicles were pretreated with LH for 3 h, then medium was removed and cells were rinsed once with medium before culturing an additional 1 to 6 h in the absence of LH. Control cells included those that were untreated or that remained in the presence of LH for the entire 9-h experimental period. All granulosa cells were collected, frozen, and subsequently processed for protein. Finally, levels of StAR mRNA in freshly collected tissue and progesterone production from incubated (for 3 h) granulosa cells derived from the largest (F1) preovulatory follicle were compared with those collected from the most recent and second-most recent postovulatory follicles (POF1 and POF2, respectively; collected approximately 10 to 12 h following the most recent preovulatory LH surge). Granulosa cell layers from postovulatory follicles were collected by gently scraping the granulosa layer away from the theca layer with a scalpel blade.

**Northern Blot Analysis**

The chicken StAR cDNA used for probing Northern blots has been previously described [5], while the chicken P450scc probe consisted of a 569-base pair polymerase chain reaction (PCR) product corresponding to nucleotides 383 to 952 from chicken P450scc (GenBank accession number D49803). Separate blots were probed for StAR mRNA or P450scc mRNA, then each was reprobed for chicken 18S ribosomal RNA to standardize for equal loading of RNA samples. Conditions for conducting Northern blot analysis have been previously detailed [21], and all blots were visualized on phosphor screens using a Storm 840 PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Analysis of blots was conducted using the ImageQuant data reduction system (Molecular Dynamics).

**Western Blot Analysis**

The StAR polyclonal antibody was generously provided by Dr. D.B. Hales (University of Illinois-Chicago; [22]). The phospho-specific Erk1/2 monoclonal antisera was from Upstate Biotechnology (Lake Placid, NY), the Erk2 polyclonal antisera was from Santa Cruz Biotechnology (Santa Cruz, CA), and the α-tubulin monoclonal antibody used for standardization was from Sigma. Western blot analyses were conducted essentially as previously described [12, 17]. Incubations with primary antibodies (phospho-Erk at a 1:1000 dilution; Erk2 at a 1:1000 dilution; and StAR at a 1:5000 dilution) were conducted overnight at 4°C, whereas those for the horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin (Ig) G secondary antibody (for phospho-Erk; Pierce, Rockford, IL) or HRP-conjugated anti-rabbit IgG secondary antibody (for Erk2 and...
**Prehierarchal Follicle Granulosa**

StAR (2.8 kb)

18S
StAR/18S

Con FSH GF GF + FSH TGFα + FSH

StAR protein

StAR (30 kDa)
tubulin
StAR/tubulin

**Preovulatory Follicle Granulosa**

Con LH Act Act CHX CHX + LH + LH

StAR (2.8 kb)

18S
StAR/18S

Con FSH GF GF + FSH TGFα + FSH

StAR protein

StAR (30 kDa)
tubulin
StAR/tubulin

![Bar charts](https://academic.oup.com/biolreprod/article/67/4/1313/2683721)

**FIG. 5.** Potentiation of FSH-induced StAR mRNA, StAR protein, and progesterone production by the PKC inhibitor GF109203X (GF; 10 μM) and the ability of TGFα to block this effect. Numbers below blots represent a relative ratio of StAR mRNA or protein to 18S rRNA or α-tubulin, respectively. Progesterone is expressed as a fold-difference compared with control (Con) cells cultured for 20 h. *P < 0.05 versus Con by paired t-test; A, B, C P < 0.05; n = 4 replicate experiments.

**FIG. 6.** Full potentiation of LH-induced progesterone production in preovulatory follicle granulosa requires de novo StAR mRNA transcription and protein translation. Granulosa cells were plated and precultured for 1 h in the absence or presence of actinomycin D (1 μg/ml) or cycloheximide (0.5 μg/ml), then subsequently cultured an additional 3 h without or with LH (100 ng/ml). Numbers below blots represent a relative ratio of StAR mRNA or protein to 18S rRNA or α-tubulin, respectively. A, B, C P < 0.05; n = 5 replicate experiments.

**RESULTS**

**Protein Kinase C Signaling in Prehierarchal Follicles**

A single 2.8-kilobase transcript for StAR mRNA was detected in whole, morphologically normal prehierarchal follicles, but it was virtually undetectable in atretic follicles (Fig. 1). As recently reported [12], the MAP kinase inhibitor, U0126, potentiates FSH-induced StAR mRNA and protein expression plus progesterone production in cultured prehierarchal follicle granulosa cells. The novel finding is that TGFα treatment attenuates this stimulatory effect on StAR expression and progesterone production (Fig. 2) even though U0126 completely prevents TGFα-induced Erk-P (Fig. 3).

Similar to the effects of TGFα, cotreatment with PMA, a pharmacologic activator of PKC, effectively blocks the induction of FSH-induced StAR mRNA and progesterone production after 20 h in culture (Fig. 4), and this inhibitory effect is not mediated via Erk-P (Fig. 3). Furthermore, pretreatment of granulosa cells with the PKC inhibitor GF109203X potentiates FSH-induced StAR mRNA and protein expression plus progesterone production. However, under these conditions, cotreatment with TGFα completely...
Granulosa cells from the largest (F1) follicle greatly exceeds that from the second (F2) plus third (F3) largest preovulatory follicles, and in both instances is associated with increased StAR protein expression. Numbers below blots represent a relative ratio of StAR protein to α-tubulin. Progesterone is expressed as a fold-difference compared to granulosa cells incubated for 30 min in the absence of LH. *P < 0.05 compared to F2+F3 granulosa; n = 3 replicate experiments.

FIG. 8. Constitutive P450scc mRNA expression in granulosa cells from F1 compared with F2 follicles. Summarized data represent P450scc levels from three different ovaries standardized to 18S rRNA. * P < 0.05 by paired t-test; n = 3 replicate experiments.

Reverses the potentiating effect of GF109203X (Fig. 5) and, importantly, GF109203X fails to attenuate TGFα-mediated Erk-P (Fig. 3).

Relationship Between StAR Expression and Progesterone Production in Preovulatory Follicles

Preculture of F2 plus F3 follicle granulosa cells with actinomycin D effectively blocks LH-induced StAR mRNA transcription and protein translation, and greatly attenuates LH-induced progesterone production after a 3-h culture (Fig. 6). By comparison, preculture with cycloheximide fails to alter LH-induced StAR mRNA, but completely inhibits any increase in StAR protein and, moreover, effectively blocks LH-induced progesterone production.

Although granulosa cells from the F1 follicle and F2 plus F3 follicles show comparable LH-induced responses after 90 to 180 min of treatment in terms of StAR protein expression, there is significantly greater progesterone production from F1 follicle granulosa cells at each interval (Fig. 7). To address this apparent discrepancy, levels of P450scc mRNA were compared in F1 versus F2 follicles, and were found to be significantly higher in granulosa cells from F1 follicles (Fig. 8).

Finally, levels of StAR protein are increased by 17.5 ± 4.4-fold after a 3-h pretreatment of cultured F1 follicle granulosa cells with LH (compared to control). Following the withdrawal of LH, StAR protein rapidly decreases to nondetectable levels within 6 h (Fig. 9). In an effort to relate this rapid loss of StAR expression to the interval following the preovulatory LH surge and early postovulatory period, in vivo, StAR mRNA (a more sensitive measure of detection than protein levels) was evaluated in freshly collected granulosa layers from the F1 and two most recent postovulatory follicles. Basal expression of the StAR transcript was readily detected in granulosa cells from the F1 follicle, but was decreased by more than 50% in granulosa cells from POF1 (collected 6 to 8 h following ovulation) and was virtually nondetectable in POF2. Similarly, basal progesterone produced after a 3-h incubation (expressed as nanograms of progesterone per micrograms of RNA) decreased from 4.9 ± 0.6 to 0.6 ± 0.1, respectively (P < 0.01; Fig. 10).

DISCUSSION

In hens, the selection of a single follicle into the final stages of growth and maturation before ovulation (the preovulatory hierarchy) occurs on a daily basis from a cohort of prehierarchical follicles that are 6–8 mm in diameter. Although the mechanism or mechanisms directly responsible for the recruitment of one prehierarchical follicle over another are not currently known, the cellular events surrounding follicle selection continue to be a focus of investigation. One immediate consequence of selection entails the differentiation of the follicle granulosa cell layer (as measured by greater StAR expression and steroidogenic potential) promoted by FSH, whereas by contrast, the fate of a follicle not selected is likely to be atresia [23].

The ability to detect StAR mRNA in whole, nonatretic prehierarchical follicles likely represents expression almost entirely within the theca layer, as granulosa cells at this stage of development express little to no StAR [5] and produce essentially no progesterone [24]. Not unexpectedly,
follicle atresia is associated with a significant decrease in LH and FSH receptor mRNA expression [13, 25] and, presumably due to the withdrawal of gonadotropin support, the loss of StAR mRNA expression. Although the theca layer from viable prehierarchal follicles is known to be steroidogenically active [26], it has not yet been established whether the loss of StAR expression, and as a result steroidogenic potential, is a contributing factor to the death of the granulosa layer or simply a result of the atretic process.

On the other hand, granulosa cells collected from normal prehierarchal follicles respond to FSH treatment with a modest but significant increase in StAR and P450scc expression plus progesterone production after 24 h of culture, yet coculture with TGFα blocks each of these effects [12, 27]. The MAP kinase inhibitors U0126 and PD98059 completely inhibit TGFα-induced Erk-P, and importantly, they potentiate FSH-induced StAR expression and progesterone production (Fig. 2; [12]). These findings support the working hypothesis that tonic MAP kinase activity prevents premature differentiation of granulosa cells and, moreover, that the inhibition of such signaling may be a prerequisite for the full potentiation of steroidogenesis following follicle selection. It is worthy of note that the ability of the MAP kinase signaling via Erk to inhibit gonadotropin-stimulated steroidogenesis in human granulosa-derived cells has recently been reported [28, 29].

However, the present data also demonstrate that even in the absence of MAP kinase signaling via Erk-P, TGFα remains capable of attenuating FSH-induced StAR expression and progesterone production (Figs. 2 and 3). Previously published data have implicated PKC signaling as a negative modulator of steroid production in hen granulosa cells [18]. The present results confirm that this negative regulation is at least in part attributed to PKC-mediated inhibition of StAR transcription (Fig. 4), and this is further supported by the finding that preculture with the pharmacologic PKC inhibitor GF109203X, greatly potentiates FSH-induced StAR expression and progesterone induction (Fig. 5). Furthermore, this effect is not mediated via MAP kinase/Erk signaling (Fig. 3). Although neither the specific PKC isoforms involved nor the precise cellular mechanisms effecting negative regulation of StAR transcription are currently known, there is evidence from mammalian model systems for the involvement of the transcription factors DAX-1 and Yin Yang 1 [1, 30].

In contrast to the inability of TGFα to completely reverse the potentiating effects of U0126 on differentiation, TGFα effectively blocks GF109203X-potentiated differentiation, presumably through the alternative MAP kinase signaling pathway. This indicates that TGFα can independently signal via both the MAP kinase/Erk-P and PKC pathways in prehierarchal follicle granulosa cells, yet suggest that MAP kinase signaling may represent the more potent differentiation-inhibiting pathway. Furthermore, unlike gonadotropin-releasing hormone in human granulosa-luteal cells [19], TGFα-induced Erk-P is not dependent on PKC signaling (Fig. 3). The findings reported herein are similar to those using cultured bovine granulosa cells from which it was concluded that TGFα inhibits PKA-mediated progesterone and estradiol production via both MAP kinase and PKC signaling [31]. We found it interesting that in the same studies, inhibition of MAP kinase signaling (using genistein) was also found to potentiate FSH-induced estradiol production. We also note that both epidermal growth...
factor and PMA have previously been found to reverse the phenotypic remodeling that occurs in porcine granulosa cells during FSH-induced differentiation and spontaneous luteinization in vitro [32, 33]. These results collectively indicate that the MAP kinase and PKC signaling pathways represent a conserved mechanism by which granulosa cell differentiation is tonically inhibited.

Once a follicle has been recruited into the preovulatory hierarchy, granulosa cells become competent to continuously secrete low levels of progesterone throughout the ovulatory cycle, plus respond to LH with a transient preovulatory surge of progesterone during the period h through 2 h before ovulation [34]. Studies have previously demonstrated that the granulosa cell layer is the primary source of progesterone production during the preovulatory LH surge, and both in vivo and in vitro data demonstrate that LH-induced progesterone production is significantly greater within F1 follicles than it is in F2 and smaller preovulatory follicles [35]. The latter observation is confirmed in the present in vitro experiment (Fig. 7). Significantly, however, the enhanced potential for progesterone synthesis is not associated with a detectable difference in LH receptor mRNA levels [13] or LH-induced StAR expression between these two stages of development, but is associated with a higher level of P450scc expression in F1 follicle granulosa cells (Fig. 8). We conclude that the increase in StAR expression in F2 follicles is necessary for rapid, LH-induced progesterone production, but is not sufficient for producing surge levels of progesterone. Instead, the data support the longstanding proposal that levels of P450scc activity [36] represent the rate-limiting step in producing the preovulatory surge of progesterone primarily by the F1 follicle granulosa layer.

In contrast to StAR expression, which increases within 90 to 180 min following gonadotropin treatment (Fig. 7, [12]), significant increases in P450scc expression occur after 8 to 16 h of treatment [27]. It is interesting to speculate why the transient preovulatory surge of progesterone induced by LH might be regulated by the product of a gene that is not rapidly regulated (e.g., P450scc) rather than one that can be acutely up-regulated (StAR). In this regard, there is evidence that progesterone, in the absence of gonadotropins, may directly affect the follicle to promote the rupture of the stigma at ovulation [37, 38]. These effects may be mediated via progesterone receptors that are known to be expressed in both the granulosa and theca layers of preovulatory follicles [39]. Therefore, from a physiologic perspective, the inability of the F2 follicle to produce sufficiently large amounts of progesterone in response to a preovulatory LH surge (due to limiting amounts of P450scc) may preclude its ability to initiate the ovulatory process, and thus ensure ovulation of only the F1 follicle.

Similar to findings derived from porcine luteinized granulosa cells [11] and mouse Leydig tumor cells [40], PKA-induced StAR expression, and as a consequence enhanced progesterone production, is largely dependent on new transcription and translation (Fig. 6). In the presence of actinomycin D, there is a marked (but not complete) reduction in LH-induced progesterone production from hen granulosa cells. This suggests that StAR protein synthesis continues in the presence of actinomycin D, and that the low but detectable levels of StAR protein are capable of facilitating limited progesterone synthesis after a 3-h culture. On the other hand, although cycloheximide does not block LH-induced StAR mRNA expression (an indication that StAR transcription does not require new protein synthesis), granulosa cells treated with cycloheximide plus LH fail to respond with either increased StAR protein expression or progesterone synthesis compared with those treated with cycloheximide alone. This latter result demonstrates the virtual requirement for new protein synthesis to mediate agonist-induced steroid production.

Following the preovulatory release of LH in vivo, serum progesterone levels decline to presurge levels by approximately 2 h before ovulation [34]. Results from the present studies suggest that this decrease in circulating progesterone is associated with a rapid loss of StAR protein, as is observed following the withdrawal of LH in cultured granulosa cells (Fig. 9). The ability of StAR protein to be rapidly turned over as described here is consistent with the previously estimated half-life of StAR mRNA (3–3.5 h, [40]). Mechanisms responsible for terminating the transient preovulatory LH and progesterone surges have yet to be adequately defined.

Unlike mammalian species in which a steroidogenically active corpus luteum forms from the remaining follicular tissues following ovulation, the postovulatory follicle of the hen rapidly becomes steroidogenically inactive and is completely resorbed within a few days to a week [41, 42]. Not unexpectedly, this decline in basal progesterone production in hen postovulatory follicles is associated with the loss of StAR mRNA expression (Fig. 10).

In summary, the data reported herein support the proposal that paracrine/autocrine factors (including but not limited to TGFα) prevent premature expression of StAR in preovulatory follicle granulosa cells by more than one receptor-mediated signaling pathway. Accordingly, in addition to ongoing efforts directed toward identifying the mechanisms by which trophic hormones promote StAR gene transcription, and by implication enhanced steroidogenic potential, emphasis should be also focused on downstream target regulatory proteins that can actively prevent StAR transcription. Subsequent to follicle selection into the preovulatory hierarchy, StAR transcription and translation can be rapidly increased within the granulosa layer in response to a preovulatory LH surge. Although greater StAR expression is clearly required to produce the preovulatory surge of circulating progesterone, it is evident that additional factors, such as levels of P450scc expression, ultimately determine the stage-dependent quantity of progesterone synthesis. Finally, in combination with the ability of StAR expression to be rapidly up-regulated by PKA signaling, the subsequent rapid loss of StAR protein following LH withdrawal provides for an ongoing, tightly controlled regulation of granulosa cell steroidogenesis.

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