BIOLOGY OF REPRODUCTION **65,** 1114–1119 (2001)

Luteal Expression of Cytochrome P450 Side-Chain Cleavage, Steroidogenic Acute Regulatory Protein, 3β-Hydroxysteroid Dehydrogenase, and 20α-Hydroxysteroid Dehydrogenase Genes in Late Pregnant Rats: Effect of Luteinizing Hormone and RU486[1]

Carlos O. Stocco,[2,4] Jorge Chedrese,[5] and Ricardo P. Deis[2,3]

Laboratorio de Reproducción y Lactancia,[4] CONICET, 5500 Mendoza, Argentina
Department of Obstetrics, Gynecology & Reproductive Sciences,[5] University of Saskatchewan, Saskatoon, Canada S7N 0W8

ABSTRACT

A decrease in serum progesterone at the end of pregnancy is essential for the induction of parturition in rats. We have previously demonstrated that LH participates in this process through: 1) inhibiting 3β-hydroxysteroid dehydrogenase (3β-HSD) activity and 2) stimulating progesterone catabolism by inducing 20α-hydroxysteroid dehydrogenase (20α-HSD) activity. The objective of this investigation was to determine the effect of LH and progesterone on the luteal expression of the steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage ($P450_{scc}$), 3β-HSD, and 20α-HSD genes. Gene expression was analyzed by Northern blot analysis 24 and 48 h after administration of LH or vehicle on Day 19 of pregnancy. StAR and 3β-HSD mRNA levels were lower in LH-treated rats than in rats administered with vehicle at both time points studied. $P450_{scc}$ mRNA levels were unaffected by LH. The 20α-HSD mRNA levels were not different between LH and control rats 24 h after treatment; however, greater expression of 20α-HSD, with respect to controls, was observed in LH-treated rats 48 h after treatment. Luteal progesterone content dropped in LH-treated rats at both time points studied, whereas serum progesterone decreased after 48 h only. In a second set of experiments, the anti-progesterone RU486 was injected intrabursally on Day 20 of pregnancy. RU486 had no effect on 3β-HSD or $P450_{scc}$ expression but increased 20α-HSD mRNA levels after 8 h treatment. In conclusion, the luteolytic effect of LH is mediated by a drop in StAR and 3β-HSD expression without effect on $P450_{scc}$ expression. We also provide the first in vivo evidence indicating that a decrease in luteal progesterone content may be an essential step toward the induction of 20α-HSD expression at the end of pregnancy in rats.

corpus luteum, luteinizing hormone, mechanisms of hormone action, ovary, pregnancy

INTRODUCTION

In all mammalian species, progesterone plays an essential role in the establishment and maintenance of pregnancy. The precise timing of both the synthesis and degradation of progesterone is crucial for reproductive success. Therefore, the expression of enzymes implicated in progesterone synthesis and catabolism need to be regulated tightly throughout pregnancy. Noteworthy, in rodents, a fall in progesterone secretion is absolutely essential for the induction of parturition [1, 2]. A biphasic decrease in serum progesterone levels has been described in rats at the end of pregnancy [3]. This biphasic pattern includes an initial, sluggish decrease followed by a sharp fall in the serum level of progesterone. Concomitant with the major drop in progesterone, an increase in the serum level of 20α-OH-progesterone, an inactive metabolite of progesterone, has been observed during both physiological and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced luteolysis [3–5]. This rapid switch in luteal steroid secretion, from progesterone to 20α-OH-progesterone, is due to an increase in 20α-hydroxysteroid dehydrogenase (20α-HSD) activity [6]. It has been shown that the increase in progesterone catabolism is due to an increase in 20α-HSD gene expression rather than to translation of existing messages or activation of existing enzymes [5, 7]. However, the mechanism responsible for the initial slow decrease in progesterone synthesis remains unknown.

Interestingly, there is evidence indicating that the expression of the 20α-HSD gene may be inhibited by progesterone [8–10], suggesting that a decrease in progesterone synthesis may be necessary for the normal expression of the 20α-HSD gene. In support of this hypothesis, we have shown that after LH administration a reduction in 3β-hydroxysteroid dehydrogenase (3β-HSD) activity occurs within 12 h and a decrease in serum progesterone levels is observed by 36 h, whereas a rise in 20α-HSD activity occurs 48 h after treatment [11]. Whether or not the change in intraluteal progesterone causes the increase in the expression or activity of 20α-HSD is presently unknown. To investigate this point in vivo, we administered a progesterone receptor antagonist, RU486, in the ovarian bursa to test whether blocking progesterone action would affect the expression of 20α-HSD. We also analyzed the effect of LH on the mRNA expression of cytochrome P450 side-chain cleavage enzyme ($P450_{scc}$), 3β-HSD, and steroidogenic acute regulatory protein (StAR). It is believed that $P450_{scc}$ and 3β-HSD are constitutively expressed in the corpus luteum [12]. However, it has consistently been found that either the application of steroidogenic stimuli or administration of luteolytic compounds (i.e., $PGF_{2\alpha}$) for long periods of time can alter the gene expression of these ste-

[1]This work was supported by grant PMT-PICT0098 from Agencia Nacional de Promoción Científica y Tecnológica and by grant PLI 325/8 PRE 023/98 from the PLACIRH (Programa Latinoamericano de Capacitación e Investigación en Reproducción Humana) to C.O.S. and grants from the Natural Science and Engineering Research Council of Canada and Canadian Institute of Health Science/Saskatchewan Health Regional Partnership to J.C.

[2]Correspondence: Carlos O. Stocco, Department of Physiology and Biophysics (M/C 901), University of Illinois at Chicago, 835 South Wolcott Avenue, Chicago, IL 60612-7342. FAX: 312 413 0159;
e-mail: costocco@uic.edu
[3]Reprint requests: Ricardo P. Deis, Laboratorio de Reproducción y Lactancia, LARLAC-CONICET, Casilla de Correo 855, 5500 Mendoza, Argentina.

Received: 23 February 2001.
First decision: 20 March 2001.
Accepted: 22 May 2001.

ISSN: 0006-3363. http://www.biolreprod.org

roidogenic enzymes [13–16]. In contrast, an acute effect on steroidogenesis could be achieved by affecting the synthesis of the StAR protein [17–19].

MATERIALS AND METHODS

Animals

Three- to four-month-old, 200–220-g, virgin female Wistar rats were kept in a controlled environment with lights-on 0600–2000 h, temperature 22–24°C, and continuous access to food and water. Under these conditions, rats generally give birth on Day 22. Vaginal smears were taken daily. Females were caged individually with fertile males on the night of proestrus. The presence of spermatozoa in the vaginal smear the following morning was designated Day 0 of pregnancy. Animal maintenance and handling were in accordance with the National Institutes of Health guide for the care and use of Laboratory Animals.

Reagents

Ovine LH (NIDDK-oLH-26) was obtained from the National Hormone and Pituitary Program, U.S. Department of Agriculture, Rockville, MD. Mifepristone (RU486) was generously provided by Russel-Uclaft (France). Bio-Max AR Film was purchased from Eastman-Kodak Co. (Rochester, NY); [α^{32}P]deoxycytidine triphosphate ([α^{32}P]dCTP) was purchased from Amersham Co. (Arlington Heights, IL). Methylcellulose (MC) and all other reagent-grade chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Experimental Procedures

To examine the effect of LH on the luteal expression of 20α-HSD, 3β-HSD type-I, cytochrome $P450_{scc}$, and StAR, pregnant rats received an s.c. administration of 10 μg of oLH or saline solution (as control) at 0800, 0900, 1000, and 1100 h on Day 19 of pregnancy. The dose of LH was determined by previous studies that demonstrated that LH induces inhibition of luteal progesterone synthesis production when administered at this time of pregnancy [8, 11]. The animals were killed 24 or 48 h after the last dose of oLH. Four to six animals were used for each treatment or the control group.

To examine the role of progesterone in the expression of 20α-HSD, 3β-HSD, and $P450_{scc}$ expression in vivo, Day-20 pregnant rats received an intrabursal injection of 3 μg per ovary of the anti-progesterone compound RU486 in 4% MC solution, to minimize leakage from the ovarian bursa. Briefly, under ether anesthesia, both ovaries were exposed through the same dorsal incision. Each animal received a bilateral intrabursal injection of 30 μl of MC (control groups) or MC plus RU486 using a Hamilton microliter syringe (705-N). Our laboratory has previously demonstrated a dual effect of RU486 throughout pregnancy on ovarian progesterone production using a similar dose and method of application [20]. The animals were killed 8 h after treatment. Four to six animals were used for each treatment or the control group.

All rats were killed by decapitation. Both ovaries were removed from each rat, trimmed from surrounding fat, and the corpora lutea rapidly isolated under a dissecting microscope in small amounts of ice-cold PBS, using an ice-cold dissecting stand. Corpora lutea were stored at −70°C until determination of mRNA levels or luteal progesterone content. All the corpora lutea from each animal were used for total RNA extraction or progesterone determination. Thus, each animal represents n = 1 for statistical analysis.

RNA Isolation and Northern Blot Analysis

Total RNA from frozen rat corpora lutea were isolated by sequential phenol, phenol-chloroform, and chloroform extraction, followed by ethanol precipitation. The RNA was quantitated and monitored for purity by evaluating the 260:280-nm absorption, whereas integrity was determined by electrophoresis. Only samples with a 260:280-nm ratio of 1.8 or higher were used. Total RNA (25 μg) was size-fractionated by electrophoresis in 1% agarose gels and transferred to nylon membranes. The following rat cDNA probes were used: 20α-HSD, type-I 3β-HSD, cytochrome $P450_{scc}$, StAR, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). These probes were generously provided by Dr. G. Gibori [21], Dr. C. Labrie [22], Dr. J. Richards [23], Dr. D. Stocco [24], and Dr. R. Wu [25], respectively. Probes were labeled by random primer extension with [α-^{32}P]dCTP (>3000 Ci/mmol; Amersham Co.) to a specific activity of 1.5–30 × 10^9 dpm/ng. Membranes were hybridized and autoradiographed as previously described [26]. In Figure 3, a slot blot was used to quantify mRNA expression; in this case RNA was loaded in the amounts of 0.5–5 ng/slot to determine the linearity of the response. Northern blot or dot blot autoradiograms were quantified by digital densitometric scanning with a Kodak Electrophoresis Documentation and Analysis System 120 (Eastman Kodak). Data were normalized relative to expression of the housekeeping gene (GAPDH). Fold values were calculated by dividing all data by the mean of the saline-treated (control) group.

TABLE 1. Luteal and serum progesterone concentrations 24 and 48 h after treatment with either saline or LH on Day 19 of pregnancy.

	Corpus luteum (pg/mg of tissue), hours after treatment		Serum (ng/ml), hours after treatment	
	24	48	24	48
Saline	34.67 ± 3.5	28.32 ± 2.2	120.43 ± 8.1	65.20 ± 7.6
LH	22.01 ± 4.1*	16.70 ± 3.5*	115.40 ± 10	35.60 ± 13*

* $P < 0.01$ versus saline.

Radioimmunoassay of Progesterone

The progesterone content of corpora lutea was measured with prior extraction according to the methodology described by Sanchez-Criado et al. [27] with slight modifications. The corpora lutea were thawed, weighed, and homogenized in 1 ml of 100% ethanol. The homogenate was centrifuged for 10 min at 2500 × *g* and the pellet extracted twice with 500 μl acetone. The combined supernatants were evaporated to dryness and redissolved in 1 ml PBS. Progesterone concentration in luteal extract or in serum was measured by an RIA developed in our laboratory [3] with an antiserum raised against progesterone-11-bovine serum albumin conjugate in rabbits. The sensitivity of the assay was less than 16 nmol/L and the inter- and intra-assay coefficients of variation were less than 10%.

Data Analysis

The relative abundance of each mRNA was quantified with a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA), normalized against levels of GAPDH mRNA in each sample, and expressed as a percentage of the control value. The significance of differences between the mean values in the control groups and LH-treated groups was determined by Student *t*-test using the Prism software package (Graph Pad Software, Inc., San Diego, CA). Differences between groups were considered statistically significant at $P < 0.05$.

RESULTS

Effects of LH on Progesterone Synthesis and Catabolism at the End of Pregnancy

Administration of LH on Day 19 of pregnancy induced a significant reduction ($P < 0.01$) in corpus luteum progesterone content 24 and 48 h after treatment when compared to control animals, whereas serum progesterone levels were only decreased after 48 h of treatment ($P < 0.01$) (Table 1).

Luteal levels of StAR mRNA were significantly lower in LH-treated animals than in saline-treated animals. A difference of 30% was observed 24 h after treatment ($P < 0.05$), whereas an 80% difference was found after 48 h of treatment ($P < 0.001$) (Fig. 1, top panel). $P450_{scc}$ mRNA levels in corpora lutea were not different between experimental and control groups either 24 or 48 h after treatment (Fig. 1, second panel from the top). However, luteal 3β-HSD mRNA levels were significantly different between experimental and control groups 24 (25%; $P < 0.01$) and 48 h (50%; $P < 0.001$) after treatment (Fig. 1, third panel from the top). Messenger RNA levels for GAPDH were not affected by LH.

The 20α-HSD mRNA levels were low and did not differ between control and LH-treated animals 24 h (Day 20) after

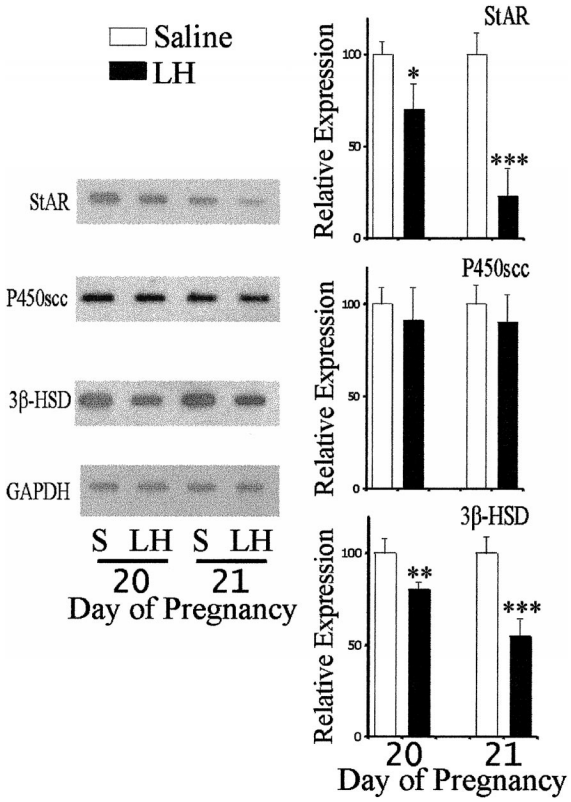

FIG. 1. Messenger RNA levels of StAR, $P450_{scc}$, and 3β-HSD in rat corpus luteum 24 and 48 h after treatment with either LH or saline (S) on Day 19 of pregnancy. Total RNA was extracted from luteal tissue and quantified by Northern blot analysis. The Northern blot is representative of four experiments. The membranes were hybridized successively with a ^{32}P-labeled cDNA probe for StAR (top), P450scc (second from the top), 3β-HSD (third from the top), or GAPDH cDNA probe (bottom). All autoradiographs were quantified by densitometric scanning. Data were normalized for GAPDH mRNA levels in each sample and expressed relative to the control value (saline). The relative amount of each mRNA in saline-treated animals was taken as 100%. The values obtained from this study as well as those from three other studies are represented (mean ± SEM, n = 4) in the bar graphs. Values with asterisks are statistically different from the control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

treatment (Fig. 2). In control animals, 20α-HSD mRNA levels were higher on Day 21 than on Day 20 of pregnancy. The 20α-HSD mRNA levels in LH-treated animals were significantly higher 48 h (Day 21) after treatment (290%; $P < 0.001$) as compared to saline-treated rats (Fig. 2).

Effect of RU486 on 20α-HSD Gene Expression

Because luteal progesterone synthesis and content decreased prior to stimulation of 20α-HSD expression, we examined whether blocking the effects of progesterone with the progesterone receptor antagonist RU486 could induce an increase in 20α-HSD mRNA levels. Eight hours after the administration of RU486 intrabursally in the morning of Day 20 of pregnancy, the expression of 20α-HSD was significantly (200%; $P < 0.001$) higher when compared to vehicle-treated animals (Fig. 3). However, the expression of 3β-HSD and $P450_{scc}$ were not different between RU486 and vehicle-treated groups, respectively (Fig. 3 and data not shown).

DISCUSSION

The results presented herein reveal that the administration of LH to rats at the end of pregnancy has a profound

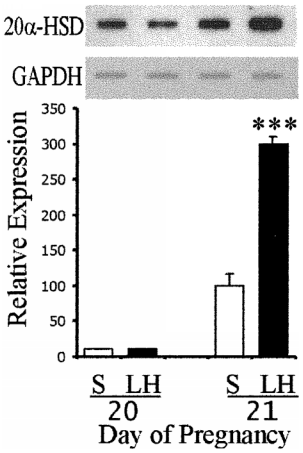

FIG. 2. Expression of 20α-HSD in rat corpus luteum 24 or 48 h after treatment with either LH or saline (S) on Day 19 of pregnancy. Total RNA was extracted from luteal tissue and quantified by Northern blot analysis. On top, a picture of a representative membrane hybridized with a ^{32}P-labeled 20α-HSD cDNA probe is shown. Next, the membrane was stripped and rehybridized with a ^{32}P-labeled GAPDH cDNA probe to control for loading. All autoradiographs were quantified by densitometric scanning. Data were normalized for GAPDH mRNA levels in each sample and expressed relative to the control value (saline). The relative amount of each mRNA in saline-treated animals was taken as 100%. The values obtained from this study as well as those from three other studies are represented (mean ± SEM, n = 4) in the bar graphs, ***$P < 0.001$.

effect on the expression of genes involved in the synthesis and catabolism of progesterone. In addition, our results show that a reduction in luteal progesterone synthesis and content may be an essential step toward the induction of 20α-HSD expression in vivo. One remarkable finding in this study is the inhibitory effect of LH on StAR expression. Previous studies have clearly demonstrated the stimulatory effect of LH and hCG on StAR [28]. To the best of our knowledge there is no previous evidence of StAR mRNA levels decreasing in response to LH. These conflicting results regarding the regulation of mRNA expression for StAR may be explained by the dual effect of LH on rat corpus luteum function. It has been demonstrated

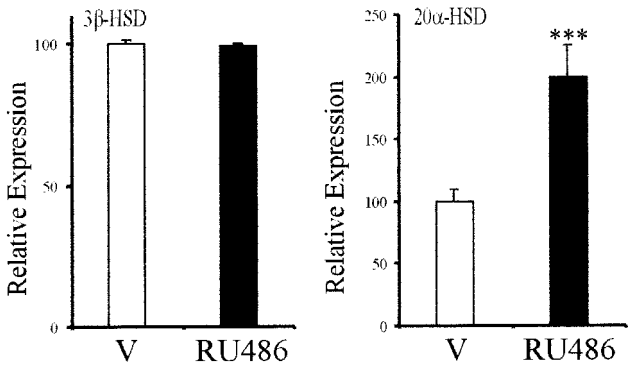

FIG. 3. Expression of 3β-HSD and 20α-HSD mRNAs in rat corpus luteum 8 h after intraovarian adminstration of RU486 or vehicle (V) on Day 20 of pregnancy. Total RNA was extracted from luteal tissue and quantified by dot-blot analysis. Membrane was stripped and then rehybridized with a ^{32}P-labeled GAPDH cDNA probe. All autoradiographs were quantified by densitometric scanning. The relative amount of each mRNA in vehicle-treated animals was taken as 100%. Data were normalized for GAPDH mRNA levels in each sample and expressed relative to the control value (vehicle). The values obtained from this study as well as those from two other studies are represented (mean ± SEM, n = 3), ***$P < 0.001$.

as early as the 1960s that LH may have different effects when administered at different times during pregnancy in rats. Thus, while the luteotrophic effect of LH during the first part of pregnancy [12] is well established, treatment with anti-LH serum from Days 14 to 18 does not affect pregnancy [29]. There is also evidence suggesting that LH may promote luteolysis in rats at the end of pregnancy [30], during the estrous cycle [31] or during lactation [32] as well as in hypophysectomized rats [33, 34]. Moreover, hypophysectomy after Day 12 of pregnancy seems to slow down the rate of fall in serum progesterone level after Day 18 [35, 36]. Sandhoff and McLean [28] have clearly demonstrated that StAR mRNA expression increases in ovaries of superovulated rats after hCG administration. Because it is clear that LH has different effects in the corpus luteum throughout pregnancy, it appears reasonable that it may have different effects on the expression of the StAR gene depending on when it is administered during pregnancy. Our results show that the switch from a luteotrophic to a luteolytic action of LH may be carried out not only through changes in its effect on the expression or activity of 3β-HSD as we have previously shown [11] but also through changes in its action on StAR gene expression (present results). It has been demonstrated that StAR mRNA and protein are absent in regressed corpora lutea [37] and are apparently down-regulated by $PGF_{2\alpha}$ [38, 39], suggesting that a decrease in StAR gene expression can also be used as a functional marker of luteolysis. Our present results demonstrate that during LH-induced luteolysis, both 3β-HSD and StAR mRNA levels are down-regulated and that this correlates with an early decline in intraluteal progesterone levels.

The conversion of cholesterol to pregnenolone by the cytochrome $P450_{scc}$ system is critical for steroid hormone synthesis and is considered the rate-limiting step for steroid hormone synthesis in adrenals and gonads. Indeed, long-term application of steroidogenic stimuli consistently alters levels of $P450_{scc}$ mRNA and protein [12]. Our results reveal that the LH-induced decrease in progesterone synthesis is not due to an effect on the expression of the $P450_{scc}$ gene.

We have demonstrated that the administration of LH on Day 19 of pregnancy results in increased 20α-HSD activity, whereas the activity of 3β-HSD is decreased [8, 11, 40]. The present results indicate that LH also induces changes in the mRNA levels of these enzymes. It is important to note that the LH-induced increase in 20α-HSD mRNA levels occurs after the reduction in intraluteal progesterone content. We have shown that LH-induced increase in 20α-HSD activity can be abolished by intrabursal administration of progesterone [8]. In addition, progesterone prevents $PGF_{2\alpha}$-induced 20α-HSD activity in the rat corpus luteum [9] and down-regulates 20α-HSD gene expression in cultured luteal cells [10]. Taken together these findings and our present results suggest that the drop in progesterone synthesis caused by LH, either by decreasing cholesterol availability to the $P450_{scc}$ system or by impairing the conversion of pregnenolone to progesterone, may trigger the increase in 20α-HSD gene expression.

The progesterone receptor is not expressed in the rat corpus luteum and is only transiently expressed in the follicle just prior to ovulation [41]. Therefore, the mechanism by which progesterone affects luteal function in the rat remains unknown. Recently, a putative mechanism whereby progesterone could regulate luteal function in the rat was proposed [10]. In a rat luteal cell line that neither expressed progesterone receptor nor produced progesterone, the presence of progesterone in the incubation media was able to down-regulate the expression of 20α-HSD mRNA in the luteal cells [10]. The authors suggested that in this case progesterone most probably was acting through a glucocorticoid receptor-mediated mechanism [10]. This mechanism is also probable in vivo because of the relatively high affinity of progesterone [42] for the glucocorticoid receptor and the high concentration reached by the steroid within the corpora lutea. The ability of RU486 to inhibit progesterone action in the corpus luteum could be due to its potential to inhibit glucocorticoid receptors at high concentrations [43]. However, additional experiments are required to determine whether the glucocorticoid receptor is involved in rat luteal cell function. Alternatively, recent evidence suggests that progesterone may act via nonclassical receptor target sites located in the plasma membrane [44]. These authors presented evidence that progesterone could mediate its anti-mitotic and anti-apoptotic effects in rat granulosa cells through a progesterone-binding protein that is localized within the surface membrane of the cells and has gamma aminobutyric $acid_A$ ($GABA_A$) receptor-like properties. The possibility that progesterone acts on luteal cells through such a receptor cannot be ruled out.

An indirect effect of progesterone on glucocorticoid catabolism may be also considered as a nonreceptor-dependent mechanism of progesterone action in the corpus luteum. It is known that progesterone inhibits the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) [45]. 11β-HSD2 regulates glucocorticoid access to its receptors by converting cortisol to its inactive metabolite cortisone [46]. It has been reported that the local inactivation of endogenous glucocorticoids within the corpus luteum (by a marked induction of 11β-HSD2 mRNA expression late in rat pregnancy) plays an important facilitatory role in the process of luteolysis [47]. Therefore it is reasonable to speculate that high levels of luteal progesterone may reduce glucocorticoid catabolism by inhibition of 11β-HSD2 activity affecting the luteolytic process.

In conclusion, our data suggest that the luteolytic effect of LH at the end of pregnancy is mediated by a reduction in cholesterol transport to the mitochondrion and a decrease in the conversion of pregnenolone to progesterone, without effect on $P450_{scc}$ gene expression. These early effects of LH result in a decrease in luteal progesterone that allows 20α-HSD expression, thus triggering the rapid decline in serum progesterone observed at the end of pregnancy. These experiments provide the first in vivo evidence indicating that a decrease in luteal progesterone content may be an essential step toward the induction of 20α-HSD expression at the end of pregnancy in rats.

ACKNOWLEDGMENTS

We thank Dr. G. Gibori, Dr. D. Stocco, Dr. Van Luu-The, and Dr. Joanne Richards for providing us with the 20α-HSD, StAR, 3β-HSD, and $P450_{scc}$ cDNAs. We also thank Jennifer M. Bowen-Shauver and Susan Ferguson-Gottschall for critical review of the manuscript. We are thankful to NIDDK for the generous supply of oLH and Russell-Uclaft for the supply of RU486.

REFERENCES

1. Bedford CA, Challis JR, Harrison FA, Heap RB. The role of oestrogens and progesterone in the onset of parturition in various species. J Reprod Fertil 1972; 16(suppl):11–23.
2. Sugimoto Y, Yamasaki A, Segi E, Tsuboi K, Aze Y, Nishimura T,

Oida H, Yoshida N, Tanaka T, Katsuyama M, Hasumoto K, Murata T, Hirata M, Ushikubi F, Negishi M, Ichikawa A, Narumiya S. Failure of parturition in mice lacking the prostaglandin F receptor. Science 1997; 277:681–683.
3. Bussmann LE, Deis RP. Studies concerning the hormonal induction of lactogenesis by prostaglandin F2α in pregnant rats. J Steroid Biochem 1979; 11:1485–1489.
4. Wiest WG, Forbes TR. Failure of 20α-hydroxy-Δ4-pregnen-3-one and 20β-hydroxy-Δ4-pregnen-3-one to maintain pregnancy in ovariectomized mice. Endocrinology 1964; 74:149–150.
5. Stocco CO, Zhong L, Sugimoto Y, Ichikawa A, Lau LF, Gibori G. Prostaglandin F2α-induced expression of 20α-hydroxysteroid dehydrogenase involves the transcription factor Nur77. J Biol Chem 2000; 275:37202–37211.
6. Wiest WG, Kidwell WR, Balogh K Jr. Progesterone catabolism in the rat ovary: a regulatory mechanism for progestational potency during pregnancy. Endocrinology 1968; 82:844–859.
7. Albarracin CT, Parmer TG, Duan WR, Nelson SE, Gibori G. Identification of a major prolactin-regulated protein as 20α-hydroxysteroid dehydrogenase: coordinate regulation of its activity, protein content, and messenger ribonucleic acid expression. Endocrinology 1994; 134: 2453–2460.
8. Stocco CO, Deis RP. Participation of intraluteal progesterone and prostaglandin F2α in LH-induced luteolysis in pregnant rat. J Endocrinol 1998; 156:253–259.
9. Telleria CM, Stocco CO, Stati AO, Deis RP. Progesterone receptor is not required for progesterone action in the rat corpus luteum of pregnancy. Steroids 1999; 64:760–766.
10. Sugino N, Telleria CM, Gibori G. Progesterone inhibits 20α-hydroxysteroid dehydrogenase expression in the rat corpus luteum through the glucocorticoid receptor. Endocrinology 1997; 138:4497–4500.
11. Stocco CO, Deis RP. Luteolytic effect of LH: inhibition of 3β-hydroxysteroid dehydrogenase and stimulation of 20α-hydroxysteroid dehydrogenase luteal activities in late pregnant rats. J Endocrinol 1996; 150:423–429.
12. Niswender GD, Nett TM. Corpus luteum and its control in infraprimated species. In: Knokil E, Neill JD (eds.), The Physiology of Reproduction, vol. 1, 2nd ed. New York: Raven Press Ltd; 1994: 781–816.
13. McGuire WJ, Juengel JL, Niswender GD. Protein kinase C second messenger system mediates the antisteroidogenic effects of prostaglandin F2α in the ovine corpus luteum in vivo. Biol Reprod 1994; 51:800–806.
14. Belfiore CJ, Hawkins DE, Wiltbank MC, Niswender GD. Regulation of cytochrome $P450_{scc}$ synthesis and activity in the ovine corpus luteum. J Steroid Biochem Mol Biol 1994; 51:283–290.
15. Tian XC, Berndtson AK, Fortune JE. Changes in levels of messenger ribonucleic acid for cytochrome P450 side-chain cleavage and 3β-hydroxysteroid dehydrogenase during prostaglandin F2α-induced luteolysis in cattle. Biol Reprod 1994; 50:349–356.
16. Hedin L, Rodgers RJ, Simpson ER, Richards JS. Changes in content of cytochrome P450(17)α, cytochrome $P450_{scc}$, and 3-hydroxy-3-methylglutaryl CoA reductase in developing rat ovarian follicles and corpora lutea: correlation with theca cell steroidogenesis. Biol Reprod 1987; 37:211–223.
17. Orly J, Stocco DM. The role of the steroidogenic acute regulatory (StAR) protein in female reproductive tissues. Horm Metab Res 1999; 31:389–398.
18. Stocco DM. An update on the mechanism of action of the steroidogenic acute regulatory (StAR) protein. Exp Clin Endocrinol Diabetes 1999; 107:229–235.
19. Reinhart AJ, Williams SC, Stocco DM. Transcriptional regulation of the StAR gene. Mol Cell Endocrinol 1999; 151:161–169.
20. Telleria CM, Deis RP. Effect of RU486 on ovarian progesterone production at pro-oestrus and during pregnancy: a possible dual regulation of the biosynthesis of progesterone. J Reprod Fertil 1994; 102: 379–384.
21. Mao J, Duan RW, Zhong L, Gibori G, Azhar S. Expression, purification and characterization of the rat luteal 20α-hydroxysteroid dehydrogenase. Endocrinology 1997; 138:182–190.
22. Zhao HF, Labrie C, Simard J, de Launoit Y, Trudel C, Martel C, Rheaume E, Dupont E, Luu-The V, Pelletier G. Characterization of rat 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase cDNAs and differential tissue-specific expression of the corresponding mRNAs in steroidogenic and peripheral tissues. J Biol Chem 1991; 266:583–593.
23. Oonk RB, Krasnow JS, Beattie WG, Richards JS. Cyclic AMP-dependent and -independent regulation of cholesterol side chain cleavage cytochrome P-450 ($P\text{-}450_{scc}$) in rat ovarian granulosa cells and corpora lutea. cDNA and deduced amino acid sequence of rat $P\text{-}450_{scc}$. J Biol Chem 1989; 264:21934–21942.
24. Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 1994; 269:28314–28322.
25. Tso JY, Sun XH, Kao TH, Reece KS, Wu R. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. Nucleic Acids Res 1985; 13:2485–2502.
26. Chedrese PJ, Zhang D, Luu The V, Labrie F, Juorio AV, Murphy BD. Regulation of mRNA expression of 3β-hydroxy-5-ene steroid dehydrogenase in porcine granulosa cells in culture: a role for the protein kinase-C pathway. Mol Endocrinol 1990; 4:1532–1538.
27. Sanchez-Criado JE, Uilenbroek JT, Karels B. Different effects of the antiprogesterone RU486 on progesterone secretion by the corpus luteum of rats with 4- and 5-day oestrous cycles. J Endocrinol 1992; 132:115–122.
28. Sandhoff TW, McLean MP. Hormonal regulation of steroidogenic acute regulatory (StAR) protein messenger ribonucleic acid expression in the rat ovary. Endocrine 1996; 4:259–267.
29. Loewit K, Badawy S, Laurence K. Alteration of corpus luteum function in the pregnant rat by antiluteinizing serum. Endocrinology 1969; 84:244–251.
30. Gordon WL, Sherwood OD. Evidence that luteinizing hormone from the maternal pituitary gland may promote antepartum release of relaxin, luteolysis, and birth in rats. Endocrinology 1982; 111:1299–1310.
31. Plas-Roser S, Muller B, Aron C. Estradiol involvement in the luteolytic action of LH during the estrous cycle in the rat. Exp Clin Endocrinol 1988; 92:145–153.
32. Yoshinaga K. Ovarian progestin secretion in lactating rats: effect of intrabursal injection of prolactin antiserum, prolactin and LH. Endocrinology 1974; 94:829–834.
33. Rothchild I. The corpus luteum-hypophysis relationship: the luteolytic effect of luteinizing hormone (LH) in the rat. Acta Endocrinol 1965; 49:107–119.
34. Rothchild I. Interrelations between progesterone and the ovary, pituitary, and central nervous system in the control of ovulation and the regulation of progesterone secretion. Vitam Horm 1965; 23:210–327.
35. Pepe G, Rothchild I. The effect of hypophysectomy on day 12 of pregnancy on the serum progesterone level and time of parturition in the rat. Endocrinology 1972; 91:1380–1385.
36. Gibori G, Richards JS. Dissociation of two distinct luteotropic effects of prolactin: regulation of luteinizing hormone-receptor content and progesterone secretion during pregnancy. Endocrinology 1978; 102: 767–774.
37. Pescador N, Soumano K, Stocco DM, Price CA, Murphy BD. Steroidogenic acute regulatory protein in bovine corpora lutea. Biol Reprod 1996; 55:485–491.
38. Sandhoff TW, McLean MP. Repression of the rat steroidogenic acute regulatory (StAR) protein gene by PGF2α is modulated by the negative transcription factor DAX-1. Endocrine 1999; 10:83–91.
39. Fiedler EP, Plouffe L Jr, Hales DB, Hales KH, Khan I. Prostaglandin F2α induces a rapid decline in progesterone production and steroidogenic acute regulatory protein expression in isolated rat corpus luteum without altering messenger ribonucleic acid expression. Biol Reprod 1999; 61:643–650.
40. Stocco CO, Deis RP. Luteinizing hormone inhibits conversion of pregnenolone to progesterone in luteal cells from rats on day 19 of pregnancy. Biol Reprod 1999; 60:729–732.
41. Park-Sarge OK, Parmer TG, Gu Y, Gibori G. Does the rat corpus luteum express the progesterone receptor gene? Endocrinology 1995; 136:1537–1543.
42. Ojasoo T, Raynaud J. Receptor binding profiles of progestins. In: Jasonni VM, Nenci I, Flamigni C (eds.), Steroids and Endometrial Cancer. New York: Raven Press Ltd.; 1983: 11–28.
43. Fryer CJ, Kinyamu HK, Rogatsky I, Garabedian MJ, Archer TK. Selective activation of the glucocorticoid receptor by steroid antagonists in human breast cancer and osteosarcoma cells. J Biol Chem 2000; 275:17771–17777.
44. Peluso JJ, Pappalardo A. Progesterone mediates its anti-mitogenic and anti-apoptotic actions in rat granulosa cells through a progesterone-binding protein with gamma aminobutyric acid A receptor-like features. Biol Reprod 1998; 58:1131–1137.

45. Quinkler M, Johanssen S, Grossmann C, Bahr V, Muller M, Oelkers W, Diederich S. Progesterone metabolism in the human kidney and inhibition of 11β-hydroxysteroid dehydrogenase type 2 by progesterone and its metabolites. J Clin Endocrinol Metab 1999; 84:4165–4171.
46. Quinkler M, Oelkers W, Diederich S. Clinical implications of glucocorticoid metabolism by 11β-hydroxysteroid dehydrogenases in target tissues. Eur J Endocrinol 2001; 144:87–97.
47. Waddell BJ, Benediktsson R, Seckl JR. 11β-Hydroxysteroid dehydrogenase type 2 in the rat corpus luteum: induction of messenger ribonucleic acid expression and bioactivity coincident with luteal regression. Endocrinology 1996; 137:5386–5391.