PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 55–61 (1987)

Effects of Cycloheximide during the Periovulatory Period on Ovarian Follicular FSH, hCG, and Prolactin Receptors and on Follicular Maturation in the Hamster (42516)

SHIOW-CHING WANG AND GILBERT S. GREENWALD[1]

Department of Physiology, Ralph L. Smith Research Center, University of Kansas Medical Center, Kansas City, Kansas 66103

Abstract. Hamsters were injected sc at 1400 hr on proestrus with either 4 mg cycloheximide (which blocks ovulation but only transiently affects ovarian protein synthesis) or saline and killed at 2-hr intervals until 0400 hr on estrus. After cycloheximide, the first surge of FSH (at 1600 hr) was half the normal value and the second surge of FSH (beginning at 2200 hr) was eliminated. Control follicles at 1400 hr had approximately the same number of FSH and hCG receptors with about one-third as many PRL receptors. Down regulation of FSH and hCG receptors for control follicles occurred by 2400 hr while PRL receptors dropped abruptly 4 hr earlier. Compared to the 1400-hr control values, the maximal loss of FSH, LH, and PRL receptors was 40, 45, and 85%, respectively. Although cycloheximide tended to slightly delay the loss of FSH receptors at 2000–2200 hr it did not prevent the ultimate fall in FSH and hCG receptors; the loss of PRL receptors was accelerated by 4 hr. Cycloheximide prevented or delayed follicular growth, resumption of meiosis, and cumulus expansion. The altered proestrous profile of steroids after cycloheximide (prolonged follicular estradiol and reduced progesterone) is therefore not associated with drastic alterations in the number of FSH and hCG binding sites. On the other hand, PRL receptors represent fast turnover protein(s).

The hamster fits the pattern of other mammals in that the gonadotropin surge at proestrus is responsible for the rise in progesterone (P_4) and the concurrent fall in estradiol-17β (E_2) and subsequent ovulation (1). A single sc injection of cycloheximide at the onset of the gonadotropin surge blunts the increase in P_4, prolongs E_2 secretion, and slightly increases serum PRL without disturbing the normal serum profiles of FSH and LH up to 2200 hr (2–5). The dose–response effects of cycloheximide on ovarian protein synthesis and follicular histology were recently described and 4 mg was the optimal dose (5). In most instances, cycloheximide blocked ovulation leading to the formation of large cystic blood-filled follicles; all hamsters survived the treatment with minimal side effects. Based on *in vitro* incubations of follicles, it appears that cycloheximide prolongs E_2 secretion in the hamster by prolonging the ability of the theca to produce androstenedione which is then available as a precursor for conversion to estrogen by the granulosal compartment (3, 4). As a follow-up to these findings, the present study was designed to determine whether 4 mg cycloheximide affects the temporal changes in the numbers of gonadotropin receptor sites on the proestrous Graafian follicle and how it acts on follicular growth and oocyte maturation. Our purpose was to use this model to further analyze what factors account for the normal shift in follicular steroidogenesis at proestrus from E_2 to P_4 dominance.

Materials and Methods. Adult female golden hamsters (Sasco Laboratories, Omaha, NE) weighing 90–120 g and with at least three consecutive 4-day cycles were used. At 1400 hr on proestrus (Day 4 of the estrous cycle), hamsters were injected sc with either 0.3 ml saline or 4 mg cycloheximide (Sigma, St. Louis, MO). Twelve animals were killed every 2 hr from 1400 hr on proestrus until 0400 hr on estrus (Day 1 of cycle) for both control and cycloheximide-treated animals. Trunk blood was saved for determination of serum FSH, LH, and PRL. For each group, to measure peptide hormone receptors, the largest healthy follicles (before ovulation) or newly ovulated follicles (after ovulation) from each of 6 animals were dissected with the aid of watchmaker's forceps, pooled for each animal, and placed in 12 × 75-mm borosilicate glass tubes

[1] To whom reprints requests should be addressed.

0037-9727/87 $1.50

containing 0.5 ml ice-cold 50 m*M* Tris buffer with 10 m*M* $CaCl_2$ and 10 m*M* $MgCl_2$ (pH 7.2). After snap freezing, the follicles were stored at −80°C until processed for receptor assays.

Ovaries from six animals of each group were fixed in Bouin's fluid, sectioned serially at 10 μm, and stained with hematoxylin and eosin. Follicular size was determined as described previously (6). Only the largest healthy antral follicles as judged by morphological criteria (6) were measured in each ovary. The process of oocyte maturation was also examined and classified.

Gonadotropin RIAs. Serum levels of prolactin (PRL), FSH, and LH were measured according to methods previously described for the hamster (7, 8) utilizing for the latter the ovine–ovine LH assay. The hormone values were expressed in terms of rat PRL-RP-1 (11 IU/mg), NIAMDD rat FSH-RP-1 (2.1 × NIH-FSH-S1), and rat LH-RP-1 (0.03 × NIH-LH-S1).

Preparation of follicular homogenates. Frozen follicles from each animal were snap thawed and homogenized using a Tissumizer (Tekmar Co., Cincinnati, OH) for 10 sec at setting 80. The homogenate was diluted to 0.5 follicle/0.1 ml with ice-cold 50 m*M* Tris buffer plus 10 m*M* $CaCl_2$ and 10 m*M* $MgCl_2$ (pH 7.2). Preliminary experiments showed that there was no difference in receptor numbers (expressed as fmole/mg protein) when the follicles were homogenized with or without 0.01% lima bean trypsin inhibitor (Sigma, St. Louis, MO). There was also no difference in receptor number (fmole/mg protein) when follicular homogenate or 20,000*g* crude follicular membrane preparation was used. Hence, in the definitive experiment follicular homogenate was used.

Preparation of radiolabeled gonadotropins. Ovine FSH (NIAMMD I-1), highly purified hCG (14500 IU/mg, Radioassay System Laboratories, Inc., Carson, CA), and ovine PRL (NIADDK, I-1) were labeled with ^{125}I (sp act. 13.9 mCi $^{125}I/\mu g$ of iodine; Amersham, IL) by the lactoperoxidase method (9). In brief, 25 μl 0.5 *M* potassium phosphate buffer (pH 7.4 for gonadotropins and pH 7.0 for PRL); 4 μg FSH, hCG, or PRL; and 60 IU lactoperoxidase (Sigma, St. Louis, MO) were added to an unstoppered ice-cooled 1-ml serum vial. A stopper was then placed on the vial and 0.3 mCi $Na^{125}I$ was injected into the vial with a Hamilton syringe. Iodination was initiated by the addition of 10 μl of a 1:80,000 dilution of 50% hydrogen peroxide (Fisher Scientific Co., Fair Lawn, NJ) into the vial. The reaction was allowed to proceed for 50 sec for FSH or hCG and 2 min for PRL and was then terminated by injection of 300 μl of 0.025 *M* Tris buffer (pH 7.2 for gonadotropins and pH 8.0 for PRL). The reaction solution was then transferred to a 0.7 × 18-cm Sephadex G-100 column and eluted with Tris buffer to separate labeled hormone from free iodine.

Characterization of radiolabeled gonadotropins. A mixture of Day 3 and Day 4 homogenates of hamsters ovaries was used for the characterization of ^{125}I-oFSH and ^{125}I-hCG while 20,000*g* crude liver membrane preparation from the Day-14 pregnant rat was used for ^{125}I-oPRL. The protein content of the receptor preparations was determined without solubilization by a protein assay kit (Bio-Rad Laboratories, Richmond, CA) using bovine serum albumin (Fraction V, Sigma, St. Louis, MO) as standard. The protein concentration in follicular homogenates solubilized with 1% SDS was 1.26 times greater than that without solubilization, with protein measured by the Lowry method. In the present experiments, however, the proteins were not solubilized because the Bio-Rad assay yields erroneous values when 1% SDS is used to solubilize the homogenates.

The active fraction and specific activity of tracer were determined by a radioligand–receptor assay (9). The active fraction of the tracers for ^{125}I-oFSH, ^{125}I-hCG, and ^{125}I-oPRL were 54, 51, and 49%, respectively, with active fraction defined as the percentage of labeled hormone which will specifically bind to an excess of its receptor. The specific activities for ^{125}I-oFSH, ^{125}I-hCG, and ^{125}I-oPRL were 29.0, 17.3, and 23.3 $\mu Ci/\mu g$, respectively, as calculated by the method of Reichert (10).

Using a preparation of hamster follicular homogenate, the optimal temperature and time required to attain binding equilibrium was 30°C for 4 to 10 h (data not shown). Therefore, 6 h was selected as the incubation period. Nonspecific binding for labeled tracers was always less than 10% of total hormone.

To evaluate temporal changes in follicular peptide hormone receptors, a fixed amount of follicular receptor preparation (equivalent to

a half follicle) from each animal was incubated with 10,000 cpm labeled peptide hormone in the presence or absence of 1000-fold excess unlabeled hormone. The amount of labeled ligand (10,000 cpm) was ascertained to be sufficient by plotting specific binding versus 0.5, 1, or 2 follicles and establishing linearity over this range. The PRL used for iodination is contaminated with less than 0.1% FSH or LH. Neither 2 μg/tube FSH nor LH demonstrates significant competition for ^{125}I-PRL binding sites in ovarian receptor preparations (11). The FSH used for iodination is contaminated with less than 0.4% LH. Excess hCG does not displace labeled FSH from sections of hamster ovaries processed for topical autoradiography (12). Specific binding of peptide hormones was expressed as femtomoles per milligram protein of follicular homogenate. Labeled hCG was used to measure LH receptor levels based on its greater stability on iodination. However, ^{125}I-labeled rat or human LH binds to the same sites as hCG in the hamster ovary (12). Therefore hCG and LH receptors will be used as interchangeable terms. The methods for the binding studies have been previously validated in our laboratory for PRL (11), hCG (13), and FSH (14).

Statistics. The data were analyzed using one-way and two-way analyses of variance and the Tukey test was applied for multiple comparisons (15). Differences were judged significant at $P < 0.05$.

Results. *Serum peptide hormone levels.* A surge in serum FSH was evident in both control and cycloheximide-treated groups at Day 4 1600 hr, but cycloheximide attenuated the magnitude of the surge ($P < 0.05$, Fig. 1A). While the second FSH surge began at Day 4 2200 hr in control animals, there was no significant increase in FSH in the cycloheximide group ($P < 0.05$). Serum LH was superimposable in both groups with the peak at Day 4 1600 hr and dropping to basal levels at Day 4 2200 hr (Fig. 1B). For both groups, serum PRL fluctuated throughout the 14-hr time span. No PRL surge was observed; however, the cycloheximide group had greater PRL levels at Day 4 1800, 2200, and 2400 hr ($P < 0.05$, Fig. 1C).

In the controls, FSH receptor numbers remained relatively constant from Day 4 1400 hr to Day 4 2000 hr, followed by a significant decrease at Day 4 2200 hr (Fig. 2). A further

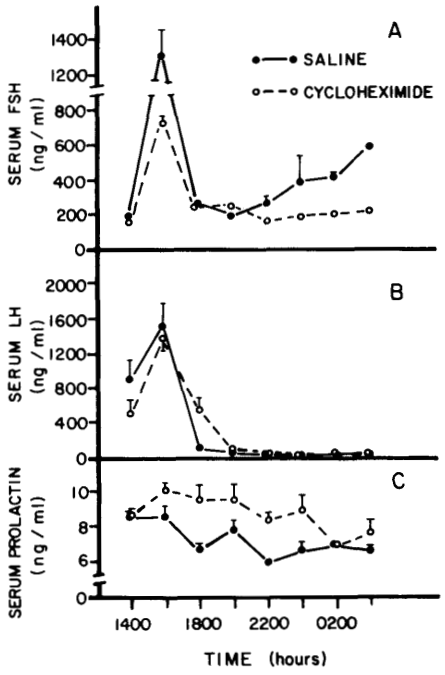

FIG. 1. Effect of cycloheximide on serum (A) FSH, (B) LH and (C) PRL levels. Hamsters were injected sc with either saline or 4 mg of cyclo at 1400 hr proestrus and killed every 2 hr until 0400 hr estrus. Identical treatments were applied in the subsequent figure. In this and the subsequent figure, data points represent means + SEM of six replicates.

decrease in FSH receptors took place between Day 1 0200 and 0400 hr—when ovulation had occurred (ovulation usually occurs at Day 1 0130 hr). After cycloheximide treatment, FSH receptors were unchanged from Day 4 1400 to 2200 hr, except for a significant decrease at 1800 hr. None of the cycloheximide-treated hamsters ovulated by Day 1 0400 hr; follicles from this group had a greater number of FSH receptors than did controls, except at Day 4 1800 hr.

The pattern of hCG receptors for control follicles was similar to that of FSH except that hCG binding sites showed no further decrease in the ruptured follicle (Fig. 2). After cycloheximide treatment, hCG receptor numbers were not significantly different at most times except that at Day 4 2000 hr, the level was significantly greater than at 1800 hr. Follicles from the cycloheximide group contained the smallest amounts of hCG receptors at Day 1 0200 and 0400 hr. In general, hCG receptor concentrations in follicles did not differ be-

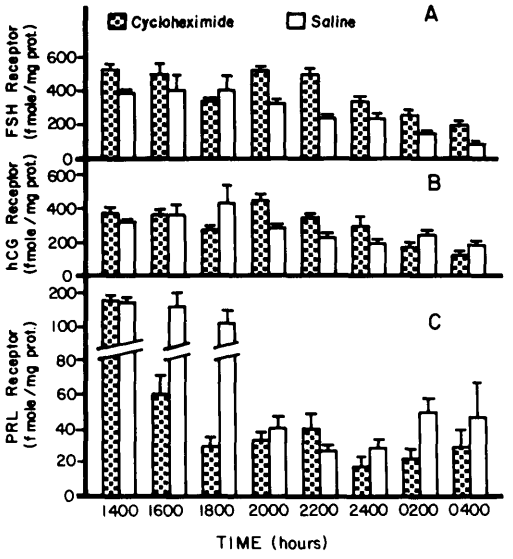

FIG. 2. Effect of cycloheximide on Graafian follicular (A) FSH, (B) hCG, and (C) PRL receptors.

tween control and cycloheximide-treated hamsters except at Day 4 2000 and 2200 hr when greater numbers were present in the latter groups.

For the controls, no significant change in PRL receptors occurred from Day 4 1400 to 1800 hr. There was an abrupt drop at Day 4 2000 hr and PRL receptors remained at this low level until Day 1 0400 hr. Cycloheximide hastened the drastic decline of PRL receptor at Day 4 1600 hr with a further fall at 1800 hr and thereafter. At most times, PRL receptor was comparable in both groups except that the control follicles had greater amounts at 1600, 1800, and 0200 hr.

Follicular growth, oocyte maturation, and cumulus expansion. For the saline-injected animals, follicular diameter increased significantly from Day 4 1400 to 1600 hr (554 ± 6 μm SEM versus 602 ± 7 μm) and was unchanged until 2000 hr (606 ± 5 μm). A significant spurt in follicular growth then occurred at 2200 hr (649 ± 10 μm) with no further change at 2400 hr. By Day 1 0200 hr, no large healthy unovulated Graafian follicle was observed; newly formed corpora lutea were present in all animals. In contrast, follicular size in the cycloheximide group was unchanged between Day 4 1400 hr and Day 1 0400 hr (573 ± 9 to 557 ± 5 μm) and no follicles had ovulated.

At Day 4 1400 hr, for both control and cycloheximide-treated follicles, the germinal vesicle was intact in all oocytes and contained nucleoli of various sizes, but 2 hr later the nuclear membrane and nucleoli became indistinct and chromatin began to condense in the control follicles. For cycloheximide-treated follicles, these events did not take place until 1800 hr. The nuclear membrane disappeared at 1800 hr in control follicles, with the majority of oocytes entering prophase I (19/26) and advancing in 7/26 oocytes to metaphase I. The nuclear membrane disappeared in 9/10 cycloheximide-treated follicles at 2000 hr; all the oocytes were in prophase I. Between Day 4 2000 and 2200 hr, 24/25 oocytes in control follicles were arrested at metaphase I, whereas only one progressed to anaphase I. By 2400 hr, 12/14 oocytes of control follicles had already extruded the first polar body and two still persisted at anaphase I. In contrast, the oocytes of cycloheximide-treated follicles remained at prophase I instead of progressing to metaphase I from Day 4 2200 hr to Day 1 0400 hr.

The oocyte in control follicles was surrounded by compact cumulus cells from 1400 to 1800 hr. By Day 4 2000 hr, cumulus cells started to disperse leaving behind a compact unilaminar ring surrounding the oocyte. The cumulus cells were even more scattered by 2200 hr. By 2400 hr, the oocyte was isolated from the membrana granulosa, and some thecal cells began to invade the granulosa layer. In contrast, for follicles exposed to cycloheximide the cumulus cells were still compact at 2000 hr. Although cumulus cells started to disperse at 2200 hr, the process was delayed. Even at Day 1 0400 hr, the arrangement of cumulus cells was similar to that of control follicles at Day 4 2000 hr.

Discussion. Monitoring the effects of 4 mg cycloheximide injected at 1400-hr proestrus at 2-hr intervals, we found that this treatment does not affect the profiles of preovulatory FSH and LH (Fig. 1). These results confirm our previous findings (5). The attenuation at 1600 h of the FSH surge by cycloheximide was not observed previously and the reason for the diminished surge in serum FSH is unknown. The disappearance of the second FSH surge and the elevation of serum PRL by cycloheximide may be due to inhibition of FSH synthesis and/or release by the hypophysis and

a nonspecific sign of stress, respectively (for details see (5)).

Peptide hormone receptors in separated theca and granulosa have been measured during the preovulatory gonadotropin surge in rat (16), sheep (17), and cow (18). The present study deals with the changes of follicular gonadotropin receptors in *intact* preovulatory follicles which have not been previously studied in any species. For control follicles in the hamster at Day 4 1400 hr (before the gonadotropin surge) the major receptors were FSH (100%) and hCG (90% of FSH numbers) whereas there were approximately 30% as many PRL receptors as FSH receptors. This is quite different from the situation for *ovarian* receptors in the proestrous rat, where LH and PRL receptors are in the majority and the number of FSH receptors is approximately 20% of the value for LH (19). Autoradiographic studies show that the binding of FSH in the hamster as well as in the rat is restricted to granulosa cells (12, 20). The species difference in gonadotropin receptors in hamster and rat might be attributed to the fact that only *follicular* receptors were measured in the hamster. This is unlikely since a similar profile of unoccupied gonadotropin receptors is evident when hamster ovarian homogenate is assayed (Wang and Greenwald, unpublished). This species difference is not unprecedented. When exposed *in vitro* to LH, the hamster follicle accumulates much higher levels of E_2 than the rat does presumably because of a much more active aromatase system (21). Could the high levels of FSH receptors in hamster granulosa cells account, in part, for this difference?

Although the cycloheximide-treated hamster had higher follicular FSH receptors at 2000 and 2200 hr than controls did there was no significant effect on follicular hCG receptors. The latter observation is especially noteworthy since cycloheximide-treated follicles *in vitro* produce androstenedione (a thecal product and precursor for E_2 synthesis by granulosa cells) for 4–6 hr longer than control hamster follicles and the theca possesses only LH receptors (12). It thus appears that cycloheximide has minor or no effects on the number of available follicular FSH and hCG/LH binding sites. Whether it perturbs the normal effects of FSH and LH by changing binding affinity to receptors requires further study. In contrast to the minor effects on FSH and hCG binding sites, cycloheximide accelerated the loss of follicular PRL receptors. Cycloheximide causes a small increase in serum PRL levels in the hamster but this elevation most likely represents a nonspecific stress response and plays no role in the subsequent disturbances in ovarian function (5). Other investigators have also found that cycloheximide rapidly reduces PRL binding sites in rat liver, adrenal, and testis in a dose-dependent manner which suggests a rapid turnover of the protein(s) associated with this receptor (22, 23).

In the rat, the preovulatory gonadotropin surge induces a 50% loss of ovarian FSH and LH receptors 4 hr later (19). Similarly, for the control hamster at 2200 hr (6 hr after the gonadotropin surge) there was a 40% reduction in FSH receptors and a 30% decline in hCG receptors (Fig. 2). Evidently, down regulation of follicular receptors in response to physiological levels of gonadotropins is much less than that in models where large amounts of hCG have been injected. In the present study, the loss of LH receptors is not attributable to the occupancy of the receptor by endogenous LH, since serum LH was very low at this time (Fig. 1). At Day 4 2200 hr—when FSH receptors decrease—the second surge of FSH is in progress. Whether this rise in FSH has any effect on the loss of FSH receptors is unknown. Whereas FSH receptors are restricted to granulosa cells, topical autoradiography reveals that hCG and PRL receptors are found in both theca and granulosa in the preovulatory hamster follicle (12).

In the present study, down regulation of follicular PRL receptor was much more precipitous and occurred 4 hr earlier than that for FSH and LH receptors. These results agree with previous findings which showed that the preovulatory LH surge is the principal cause of the drastic loss of PRL receptor (11). Injection of 4 mg cycloheximide at 1400 hr proestrus reduces ovarian protein synthesis at 1600 and 2200 hr to 37 and 65%, respectively, of control values (5). Hence, the effects of cycloheximide are beginning to wear off by 2200 hr. The reduced protein synthesis may account for the more rapid decline in PRL receptors but does not correlate with any changes in FSH or hCG receptors.

The diameter of the control follicle on proestrus between 1800 and 2400 hr is similar to previous reports (6) which ascribed the

growth to an increase in liquor folliculi. The effects of gonadotropins and steroids on the stimulation of mucopolysaccharide synthesis has been reported to be causally related to cumulus expansion and hence follicular size (for references see (24)). Since cycloheximide is a potent protein synthesis inhibitor, it may delay cumulus expansion and arrest follicular growth simply by blocking mucopolysaccharide synthesis by the granulosa compartment. Although cycloheximide greatly retards follicular development up to Day 1 0400 hr, by 0900 hr normal preovulatory growth is restored (5) presumably as its lingering effects wear off.

Although serum PRL in the cycloheximide group is greater than that in control values (Fig. 1) this slight elevation hardly accounts for the delay of oocyte maturation. It is possible that postponement of the decline of E_2 in the cycloheximide group delays oocyte maturation. Alternatively, the *de novo* appearance of microtubule-associated proteins may be blocked directly by cycloheximide, thus preventing the development of the spindle apparatus.

In summary, cycloheximide does not act by altering FSH and LH binding sites but may change the conformation or accelerate the loss of PRL receptors. It also impairs follicular growth, resumption of meiosis, and cumulus expansion. Based on our previous findings, when cycloheximide is injected at the onset of the LH surge, its major influences on steroidogenesis are (i) to prolong the ability of the theca to produce androgen precursors for conversion to estrogens by granulosa cells (4) and (ii) concurrently to block the conversion of cholesterol to C-21 steroids by the granulosa compartment. As shown in this paper, changes in FSH and hCG/LH receptor numbers are evidently not involved in the altered steroidogenic profiles following *in vivo* treatment with cycloheximide. We have recently been able to simulate the *in vivo* follicular changes elicited by cycloheximide by its direct action *in vitro* (Wang and Greenwald, unpublished). Hence, stress-related nonspecific events seem to be eliminated as confounding factors in this model of preovulatory hormonal changes.

This work was supported by a grant from NIH (HD-00596). We thank the National Hormone and Pituitary Program (NIADDK) for providing the hormones.

1. Saidapur SK, Greenwald GS. Regulation of 17β-estradiol synthesis in the proestrous hamster: Role of progesterone and luteinizing hormone. Endocrinology **105**:1432–1439, 1979.
2. Saidapur SK, Greenwald GS. The role of protein synthesis in regulation of oestradiol-17β in the pro-oestrous hamster. J Reprod Fertil **62**:379–384, 1981.
3. Greenwald GS, Limback D. Effects of treatment with cycloheximide at proestrus on subsequent in vitro follicular steroidogenesis in the hamster. Biol Reprod **30**:1105–1116, 1984.
4. Wang SC, Greenwald GS. Alterations in follicular function in the proestrous hamster by the injection of cycloheximide. In: Toft DO, Ryan RJ, Ed. Proceedings, 5th Ovarian Workshop. Champaign, Illinois: Ovarian Workshop, p189, 1985.
5. Wang SC, Greenwald GS. Effect of cycloheximide injected at proestrus on ovarian protein synthesis, peptide and steroid hormone levels and ovulation in the hamster. Biol Reprod **33**:201–211, 1985.
6. Norman RL, Greenwald GS. Follicular histology and physiological correlates in the preovulatory hamster. Anat Rec **173**:95–108, 1972.
7. Bast JD, Greenwald GS. Serum profiles of follicle-stimulating hormone, luteinizing hormone and prolactin during the estrous cycle of the hamster. Endocrinology **94**:1295–1299, 1974.
8. Siegel HI, Bast JD, Greenwald GS. The effects of phenobarbital and gonadal steroids on periovulatory serum levels of luteinizing hormone and follicle-stimulating hormone in the hamster. Endocrinology **98**: 48–55, 1976.
9. Catt KJ, Ketelslegers J, Dufau ML. Receptors for gonadotropic hormones. In: Blecher M, Ed. Methods in Receptor Research. New York: Dekker, Part I, pp175–250, 1976.
10. Reichert LE Jr. Follicle-stimulating hormone: Measurement by a rat testis tubule tissue receptor assay. In: Blecher M, Ed. Methods in Receptor Research. New York: Dekker, Part I, pp99–118, 1976.
11. Oxberry BA, Greenwald GS. Down regulation of prolactin receptors in the hamster ovary by the preovulatory gonadotropin surge. Biol Reprod **31**:464–470, 1984.
12. Oxberry BA, Greenwald GS. An autoradiographic study of the binding of ^{125}I-labeled follicle-stimulating hormone, human chorionic gonadotropin and prolactin to the hamster ovary throughout the estrous cycle. Biol Reprod **27**:505–516, 1982.
13. Kim I, Greenwald GS. Evidence for rapid loss of spare hCG receptors in the corpora lutea of the hypophysectomized rat. Mol Cell Endocrinol **40**:123–128, 1985.
14. Kim I, Greenwald GS. Occupied and unoccupied FSH receptors in follicles of cyclic, hypophysectomized or hypophysectomized/gonadotropin-treated hamsters. Mol Cell Endocrinol **44**:141–145, 1986.
15. Winer BF. Statistical principles in experimental design, 2nd ed. New York, McGraw-Hill, 1971.

16. Uilenbroek JTJ, Richards JS. Ovarian follicular development during the rat estrous cycle: Gonadotropin receptors and follicular responsiveness, Biol Reprod **20**:1159–1165, 1979.
17. Webb R, England BG. Identification of the ovulatory follicle in the ewe: Associated changes in follicular size, thecal and granulosa cell luteinizing hormone receptors, antral fluid steroids, and circulating hormones during the preovulatory period. Endocrinology **110**:873–881, 1982.
18. Ireland JJ, Roche JF. Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. Endocrinology **111**: 2077–2086, 1982.
19. Solano AR, Vela AG, Catt KJ, Dufau ML. Regulation of ovarian gonadotropin receptors and LH bioactivity during the estrous cycle. FEBS Lett **122**:184–188, 1980.
20. Midgley AR. Autoradiographic analysis of gonadotropin binding to rat ovarian tissue sections. Adv Exp Med Biol **36**:365–378, 1973.
21. Wang SC, Greenwald GS. Effect of lipoproteins, 25-hydroxycholesterol and luteinizing hormone on in vitro follicular steroidogenesis in the hamster and rat. Biol Reprod **31**:271–279, 1984.
22. Kelly PA, Posner BI, Friesen HG. Effects of hypophysectomy, ovariectomy, and cycloheximide on specific binding sites for lactogenic hormones in rat liver. Endocrinology **97**:1408–1415, 1975.
23. Katikineni M, Davies TF, Catt KJ. Regulation of adrenal and testicular prolactin receptors by adrenocorticotropin and luteinizing hormone. Endocrinology **108**:2367–2374, 1981.
24. Eppig JJ. Regulation of cumulus oophorus expansion by gonadotropins in vivo and in vitro. Biol Reprod **23**:545–552, 1980.

Received December 24, 1985. P.S.E.B.M. 1987, Vol. 185.
Accepted January 21, 1987.