PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 537–540 (1975)

Modulation of the Uptake and Retention of Estradiol-17β in the Ovine Corpus Luteum by Luteinizing Hormone[1] (38845)

P. J. SHERIDAN,[2] J. L. PHILLIPS,[3] K. R. SIMMONS,[4] J. L. CAFFREY,[5] J. H. ABEL, AND G. D. NISWENDER

Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80523

It is clear that both luteinizing hormone (LH) and prolactin are luteotropic in the ewe (1, 2). It is also known that exogenous prostaglandin (PG) $F_{2\alpha}$ causes luteal regression in this species (3). However, attempts to explain the formation, maintenance, and regression of the ovine corpus luteum in terms of LH, prolactin, and prostaglandins have been unsuccessful (4). Recent data (5–11) strongly suggest that estrogen may affect the corpus luteum directly, or by modulating the activity of other hormones which influence luteal function, namely, LH and prolactin, or by modulating the action of prostaglandins.

Exogenous estrogens were reported to have both luteolytic and luteotropic effects in the ewe (5–9), while the removal of endogenous estrogens by destruction of ovarian follicles was associated with luteal maintenance (10, 11). The luteotropic effect of estrogen was attributed to the release of LH from the pituitary (8, 9). The luteolytic effect was ascribed to stimulation of uterine prostaglandin synthesis and release (12), since the luteolytic effect required the presence of the uterus (5, 13).

The possibility of a direct effect of estrogen on the ovine corpus luteum was suggested when the injection of estrogen directly into the corpus luteum resulted in luteal regression (14). In addition, the cytosol of luteal cell contains a specific binding protein for estradiol (15). The studies described in this communication were conducted to determine if estradiol-17β is taken up and retained by the ovine corpus luteum and to determine if the uptake and retention of estradiol varied during the estrous cycle. Since uptake did vary at different stages of the cycle, we also determined whether the uptake was influenced directly by the pituitary gonadotropins.

Materials and Methods. Superovulation. Ewes were implanted with progesterone for 20 days and received 1000 IU of pregnant mare serum gonadotropin (PMS) on the day the implant of progesterone was removed. Thirty-six hours after the injection of PMS, the ewes were treated with 500–1000 IU of human chorionic gonadotropin (HCG). The day on which HCG was injected was defined as day 0.

Cell preparation. Coprora lutea were removed from ewes on selected days after the HCG injection. Corpora lutea were sliced, rinsed, and incubated at 36° in Medium 199 containing collagenase (2000 units/gram). When under microscopic examination of the medium, free luteal cells were first detected, medium and enzyme were renewed, and the incubation was continued until only free cells were present (about 2 hr). The contents of the flasks were then centrifuged, the supernatant was discarded, and the pellet was resuspended in fresh medium. This procedure was repeated three times. The final suspension was evaluated for cell number and viability and diluted to approximately 1.2×10^6 total cells per ml.

Binding of estradiol. Estradiol-17β-2,4,6,7-^{3}H (.33 ng) in 0.1 ml of 10% ethanol–Medium 199 was added to incubation flasks containing 1.2×10^6 cells in 1 ml Medium 199. Nonspecific binding (background) was evaluated by adding 100-fold excess (33 ng) of nonradioactive estradiol-17β. The flasks were incubated at 36° for different times, and

[1] This research was supported in part by a grant from the Rockefeller Foundation and NIH Contract 69-2134.

[2] NIH Postdoctoral Fellow No. 1F22 HD01309-01.

[3] Rockefeller Postdoctoral Fellow.

[4] Present address: Department of Animal Sciences, University of Vermont, Burlington, Vermont.

[5] Rockefeller Postdoctoral Fellow.

their contents were poured into Millipore Manifolds containing GFA filters and rinsed three times with 5 ml phosphate (0.01 *M*)-buffered saline (0.14 *M*) (PBS) at pH 7.0. The filters were removed and placed in scintillation vials containing 15 ml Triton X 100 scintillation fluid (2 toluene: 500 mg dimethyl POPOP: 168 Permaflour: 1 Triton X 100). The samples were counted; specific binding was defined as the total counts associated with the cells minus the counts associated with the cells in the presence of a 100-fold excess of nonradioactive estradiol-17β.

Results. In preliminary experiments, the specific uptake of ^{3}H-estradiol reached a maximum in 5 min and plateaued for 1 hr. Further incubations were carried out for 8 min. When the radioactivity taken up by cells obtained by midcycle was extracted and subjected to chromatography on Sephadex LH-20 columns, over 90% of the radioactivity eluted as estradiol-17β.

To determine the specificity of uptake, cells were incubated in the presence of a 10-fold excess (3.3 ng) of nonradioactive steroids. The percentage inhibition of uptake of ^{3}H-estradiol-17β was determined for: estradiol-17β 69% inhibition, estrone 41% inhibition, estriol 0% (a slight stimulation was observed), estradiol-17α 0% (a slight stimulation was observed), progesterone 21% and testosterone 31%.

The uptake and retention of ^{3}H-estradiol varied during the cycle (Fig. 1). During the first 2–4 days of the cycle, the uptake and retention was low; maximum uptake and retention was observed between days 8 and 12 of the cycle. The uptake and retention were significantly ($P < 0.05$) reduced on days 13 and 14 then increased on day 15.

To determine if LH, FSH, and prolactin would affect the uptake and retention of ^{3}H-estradiol, cells were incubated in the presence of the three gonadotropins. LH (10 ng and 100 ng of NIH-LH-S18) inhibited ($P < 0.05$) the uptake of estradiol on days 9–12 of the cycle (Fig. 2); however, no effect of LH was demonstrated during the first few days (2–5) of the cycle nor at the end of the cycle (day 15). No effect of FSH (100 ng of NIH-FSH-S10) or prolactin (100 ng of NIH-P-S10) was noted at any time during the cycle.

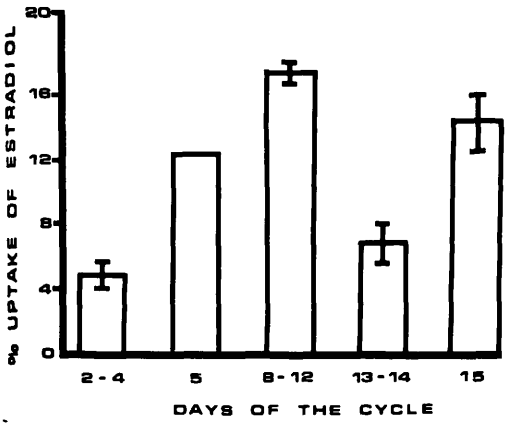

FIG. 1. The specific uptake of ^{3}H-estradiol-17β by cells of the corpus luteum of the ewe varies as a function of the estrous cycle. Plotted here is the percentage uptake as a function of the days of the cycle. Values given are means and standard errors except for day 5 on which only one experiment with three replicates was run.

Since LH inhibited estradiol uptake at times when LH maximally stimulated progesterone synthesis in the cell suspensions and since progesterone inhibited estradiol uptake, a study was designed to determine whether LH stimulation of progesterone synthesis was necessary for LH to inhibit estradiol uptake. Cells were incubated with or without LH and with or without aminoglutethimide (25–200 μg/ml). Both estradiol uptake and progesterone secretion were monitored. It is shown in Fig. 3 that when LH stimulates progesterone synthesis, it also inhibits estradiol uptake, and when the stimulation of progesterone synthesis by LH is blocked, there is no effect of LH on the uptake of estradiol. Thus, it appears that the inhibition of uptake of estradiol by LH is mediated through the stimulation of progesterone synthesis.

Discussion. These studies demonstrate a specific uptake and retention of ^{3}H-estradiol-17β by isolated luteal cells from ewes, which varies as a function of the stage of the estrous cycle. Progesterone and testosterone inhibited the uptake of ^{3}H-estradiol in contrast to data reported by others using homogenates of luteal tissue (15). When aminoglutethimide was used to block the stimulation of progesterone synthesis by LH, LH no longer inhibited the uptake of estradiol,

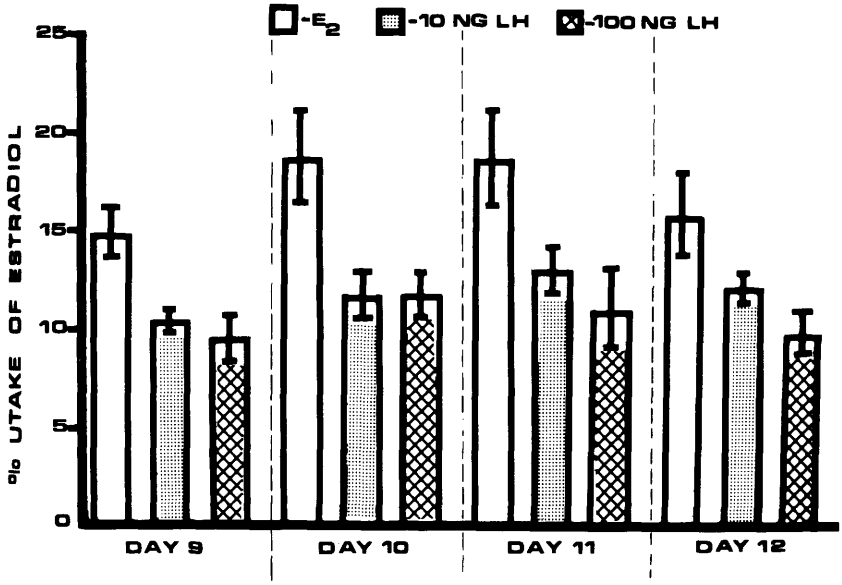

FIG. 2. Luteinizing hormone (LH) inhibits the uptake of ^{3}H-estradiol by cells of the corpus luteum of the ewe on days 9–12 of the cycle. Values given are means and standard errors.

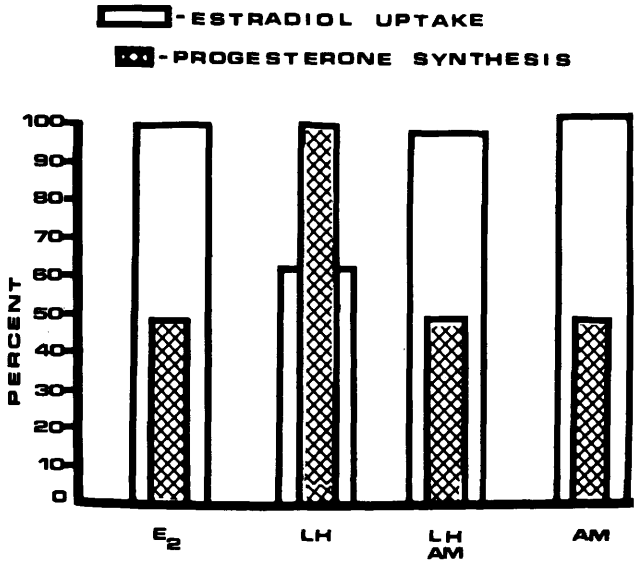

FIG. 3. Aminoglutethimide blocks the inhibition of ^{3}H-estradiol-17β uptake by luteinizing hormone (LH) and also blocks LH stimulation of progesterone synthesis.

further illustrating the ability of progesterone to inhibit the uptake of ^{3}H-estradiol. To our knowledge, the only previous report of the inhibition of estradiol uptake by progesterone was when the binding of estradiol was studied *in vivo* in the oviduct of the rhesus monkey (17).

These studies suggest that the uptake and retention of estradiol by ovine luteal cell may be modulated by levels of progesterone and indirectly by levels of LH. These findings, along with the finding that prostaglandin $F_{2\alpha}$ is unable to induce luteolysis in ewes after destruction of the follicles by X-irradiation (18), and the findings that injections of estrogen directly into the ovine corpus lu-

teum induce regression suggest that luteal regression in this species is the result of a complex interaction between estradiol, prostaglandin $F_{2\alpha}$, progesterone and LH.

Summary. The uptake and retention of ^{3}H-estradiol by the ovine corpus luteum (induced by superovulation) was maximal through days 8–12 of the cycle with little or no uptake and retention during luteinization or regression. LH, but not FSH or prolactin, inhibited the uptake and retention of estradiol via stimulation of progesterone synthesis.

Progesterone-containing implants were generously provided by Dr. R. E. Mauer, Abbott Laboratories. PMS was generously supplied by the Hormone Distribution Program of NIH and Ayerst Laboratories. HCG-Gonamone was generously supplied by the Fort Dodge Laboratories, Inc. LH, FSH and Prolactin were kindly supplied by the NIH Hormone Distribution Program. Aminoglutethimide was generously supplied by Dr. J. J. Chart, Ciba-Geigy.

1. Schroff, C., J. M. Klindt, C. C. Kaltenbach, J. W. Graber, and G. D. Niswender, J. Anim. Sci. **33,** 268 (1971).
2. Denamur, R., J. Martinet, and R. V. Short, J. Reprod. Fert. **32,** 207 (1973).
3. Goding, J. R., *in* Collogue de la Societe National L'etude de la Sterilite et del la Fecondite. Masson, Editeurs, Paris (1973).
4. Akbar, A. M., T. M. Nett, and G. D. Niswender, Endocrinology **94,** 1318 (1974).
5. Stormshak, F., H. E. Kelley, and H. W. Hawk, J. Anim. Sci. **29,** 476 (1969).
6. Hawk, H. W., and D. J. Bolt, Biol. Reprod. **2,** 275 (1970).
7. Denamur, R., J. Martinet, and R. V. Short, J. Reprod. Fert. **23,** 109 (1970).
8. Howland, B. E., A. M. Akbar, and F. Stormshak, Biol. Reprod. **5,** 25 (1971).
9. Bolt, D. J., H. E. Kelley, and H. W. Hawk, J. Anim. Sci. **32,** 977 (1971).
10. Karsch, F. J., J. W. Noveroske, J. F. Roche, H. W. Norton, and A. V. Nalbandov, Endocrinology **87,** 1228 (1970).
11. Ginther, O. J., An. J. Vet. Res. **32,** 59 (1971).
12. Caldwell, B. V., S. A. Tillson, W. A. Brock, and L. Speroff, Prostaglandins **1,** 217 (1972).
13. Bolt, D. J. and H. W. Hawk, J. Anim. Sci. **35,** 237 (1972).
14. Cook, B., F. J. Karch, D. L. Foster, and A. V. Nalbandov, Endocrinology **94,** 1197 (1974).
15. Cook, B. and T. Taylor, Seventh Annual Meeting of the Society for the Study of Reproduction, Ottawa, 1974, Abstr. 50.
16. Simmons, K. R., J. L. Caffrey, J. L. Phillips, J. H. Abel, and G. D. Niswender, Submitted to Biol. of Reprod.
17. Brenner, R. M., J. A. Resko, and N. B. West, Endocrinology **95,** 1094 (1974).
18. Hixon, J. E., D. R. Gengenbach, and W. Hansel. Seventh Annual Meeting of the Society for the Study of Reproduction, Ottawa, 1974, Abstr. 9.

Received Jan. 20, 1975. P.S.E.B.M., 1975, Vol. 149.