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Differential Steroidogenic Responses of Ovine Luteal Cells to Ovine Luteinizing Hormone and Human Chorionic Gonadotropin[1] (41824)

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Abstract. Experiments were conducted *in vitro* on ovine small luteal cells to evaluate their steroidogenic response to ovine luteinizing hormone (oLH) and human chorionic gonadotropin (hCG) administered continuously throughout the experimental period or as a 15-min pulse. Both oLH and hCG stimulated a significant increase in progesterone secretion ($P < 0.001$) by small luteal cells. Human chorionic gonadotropin administered continuously or as a pulse maintained progesterone secretion at 40–55% of experimental maximum at least 6 hr while oLH-stimulated progesterone secretion declined to basal levels by 4 hr after a 15-min pulse or declined to 25% of the experimental maximum within 6 hr under constant stimulation. The responses of small luteal cells to oLH and hCG were found to differ ($P < 0.001$). The sustained progesterone secretion of luteal cells in response to a pulse of hCG may be due to longer residence of occupied receptor complex on the cell membrane. In contrast, the decline in oLH stimulated progesterone secretion, even when hormone is continuously present in the medium, may be related to a rapid internalization of receptor–hormone complexes and down-regulation of receptors.

Human chorionic gonadotropin (hCG) is frequently used in studies of gonadotropin stimulated steroidogenesis and receptor-mediated endocytosis (1–3). The primary reasons for using hCG instead of luteinizing hormone (LH) are that both hormones stimulate steroidogenesis by binding to the same receptor, hCG can be radioiodinated to a higher specific activity with retention of its ability to bind to receptor, ^{125}I-hCG is more stable than ^{125}I-LH upon storage, and less nonspecific binding is found with hCG (1). However, the assumption that similar steroidogenic responses will be elicited in target cells by LH and hCG may be incorrect.

Segaloff *et al.* (4) investigated the steroidogenic responses of perfused Leydig tumor cells to both hCG and oLH. Their study demonstrated that a 10-min pulse of hCG (maximum stimulating dose) produced a response similar to that obtained during continuous perfusion of this hormone. However, a pulse of oLH (maximum stimulating dose) elicited a response of much shorter duration. Thus, there appears to be a difference in the steroidogenic response in Leydig tumor cells when hCG is compared to oLH. A similar effect was obtained with normal Leydig cells. Similar information is not available for other target tissues such as the corpus luteum. We, therefore, investigated progesterone secretion by LH responsive ovine small luteal cells (5) in response to different doses of oLH and hCG administered as a 15-min pulse or continuous challenge.

Materials and Methods. Experiments were performed on small luteal cells (8–18 μm diameter) obtained from ovine corpora lutea on Day 10 of the luteal phase during the 1982–1983 breeding season. The method for isolation of small luteal cells has been described (5). Briefly, corpora lutea were collected from superovulated ewes 11 days following hCG administration (6). Corpora lutea were obtained from two to seven ewes for each experiment and placed into Medium 199 (GIBCO, Richmond, Calif.; containing 4.2 m*M* $NaHCO_3$, 20 m*M* Hepes, 100 U penicillin G/ml, 100 μg streptomycin sulfate/ml, and 50 μg neomycin/ml, pH 7.35). After decapsulation, slices (0.5 mm thick) were prepared with a Stadie–Riggs hand microtome. A suspension of single cells was prepared by dissociation of these slices in Ca- and Mg-free Hanks' balanced salt solution (20 m*M* Hepes, 100 U penicillin G/ml, 100 μg streptomycin sulfate/ml, 1.0% bovine serum albumin fraction V, pH 7.35, and 2000 U collagenase/g tissue/flask), 4–5 g tissue/10 ml/flask (3), and

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0.02% deoxyribonuclease was added to inhibit cell reaggregation.

Large (>18 μm diameter) and small luteal cells were then separated using a Beckman JE-6 elutriator rotor equipped with a Sanderson chamber (5). Fractions containing small luteal cells were devoid of large cell contamination. Concentration and purity of the small luteal cell fraction was assessed with the aid of a hemocytometer and an ocular micrometer.

Small luteal cells were allowed to attach overnight (~18 hr) to 35 × 10-mm sterile tissue culture dishes (Costar, Cambridge, Mass.; 200,000 cells/dish) in 2 ml of Medium 199 (containing 2% ram serum) at 37°C in air. Culture dishes were randomly assigned to treatments in triplicate: (i) Control (no hormone stimulation); (ii) oLH (NIAMMD-oLH-22) at 1, 10, and 100 ng/ml; and (iii) hCG (CR121, 13,450 IU/mg) at 0.2, 2, and 20 ng/ml. Each hormone was administered either as a 15-min pulse or continuously throughout the 6-hr incubation period. Each experiment was replicated on two different days.

Prior to the start of an experiment each plate was washed five times with 2 ml Medium 199 containing 2% ram serum. All incubations were carried out at 37°C in air. To establish a baseline of progesterone secretion, medium in all dishes was exchanged every 15 min for 1 hr prior to hormone addition and, thereafter, every 15 min for an additional 6 hr. Control and pulse treatment cells were washed four times with 2 ml of Medium 199 (containing 2% ram serum) following the 15-min exposure to hormone to remove any residual free hormone.

At the termination of each experiment cell viability was determined by ethidium bromide/acridine orange fluorescence (7). Cell number per dish was calculated by counting all cells within five regions of the culture dish which had a known surface area. The culture medium was pooled over 30-min intervals and the progesterone concentration in the unextracted medium was determined by radioimmunoassay (8). Since the basal levels of progesterone secreted varied from 0.5 to 7.6 ng/ml between experiments, the progesterone concentrations were transformed to a percentage of the maximum response within each experiment with a maximum response equal to 100%. Analysis of variance was performed using the average response of triplicates within treatments. There was no difference ($P > 0.1$) between the data obtained with the two highest doses of either hormone within treatments, therefore, these data were pooled within similar experiments.

Results. Data regarding the responses of small luteal cells to oLH and hCG are shown in Figs. 1 and 2, respectively. Control cells maintained progesterone secretion at 7.3 ± 3.1% (mean ± SD) of the experimental maximum for the duration of each experiment. Viability of luteal cells at the end of the experiment was greater than 95% for all treatment groups in all experiments, and a significant loss of cells did not occur.

Maximum progesterone secretion was obtained 1 hr following the addition of 10 ng/ml oLH, whether administered as a 15-min pulse or continuously. Thereafter, the amount of progesterone in the medium declined to

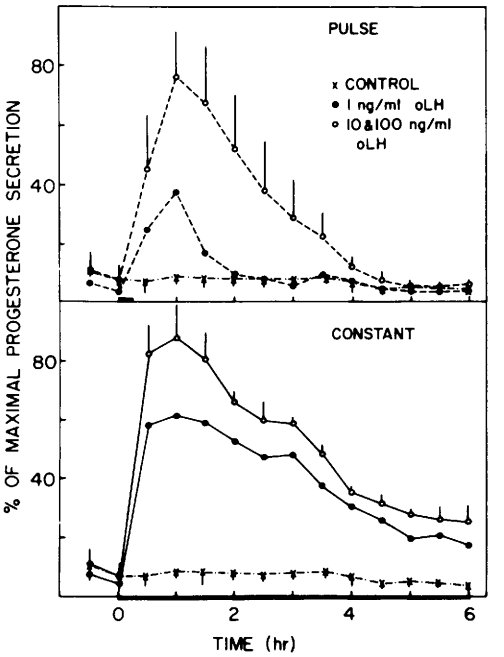

FIG. 1. Progesterone levels in medium after exposure of small luteal cells to oLH. Progesterone levels presented as percentage of maximal progesterone secretion (mean ± SD) (ordinate) by time in hours (abscissa). Bold bar along abscissa shows duration of exposure of cells to hormone. The dose of 1 ng/ml was used in a single experiment. Basal progesterone secretion varied from 1.3 to 7.6 ng/ml and peak secretion from 33.6 to 61.9 ng/ml.

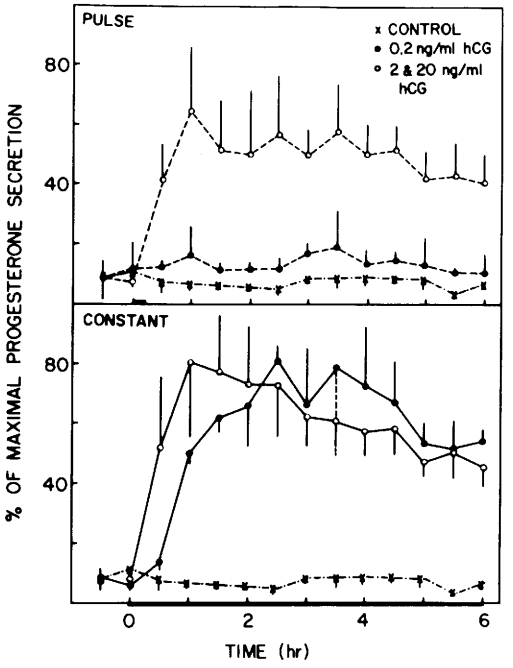

FIG. 2. Progesterone levels in medium after exposure of small luteal cells to hCG. Progesterone levels presented as percentage of maximal progesterone secretion (mean ± SD) (ordinate) by time in hours (abscissa). Bold bar along abscissa shows duration of exposure of cells to hormone. Basal progesterone secretion varied from 0.5 to 3.5 ng/ml and peak secretion from 19.4 to 21.3 ng/ml.

basal levels by 4 hr after pulse treatment, but was still elevated at 6 hr (25.1 ± 5.6% of experimental maximum) following continuous treatment.

A 1-ng/ml pulse of oLH resulted in an increase ($P < 0.001$) in progesterone levels which returned to basal levels by 2 hr. However, continuous stimulation with 1 ng/ml oLH produced a response that was similar to the 10- and 100-ng/ml treatments ($P > 0.1$) which had not returned to basal levels by 6 hr.

A small but significant ($P < 0.001$) increase in progesterone secretion was obtained with a 15-min pulse of 0.2 ng/ml hCG. Maximum progesterone secretion was obtained with 2 ng/ml hCG when administered as a 15-min pulse ($P < 0.001$).

Continuous treatment with hCG at any of the three doses elicited an increase in progesterone secretion ($P < 0.001$). The higher doses of hCG maintained progesterone secretion at 40–55% of the experimental maximum for at least 6 hr. In addition, there was no difference ($P > 0.1$) in the responses of luteal cells to either 2 or 20 ng/ml hCG when administered either as a pulse or continuously.

Luteal cells exposed to a maximum stimulating dose of either oLH or hCG exhibited a similar initial response, that is, an initial increase in progesterone secretion which was maximal by 1 hr. After reaching maximum levels, oLH stimulated progesterone secretion declined steadily while after stimulation by hCG progesterone secretion remained elevated. This response pattern was different ($P < 0.001$) for the two hormones.

Discussion. Results from the present study with ovine luteal cells were similar to those obtained with Leydig cells by Segaloff *et al.* (4). A short pulse of hCG resulted in prolonged secretion of steroid, comparable to that obtained with a constant treatment while short-term treatment with oLH stimulated steroidogenesis only briefly. The reasons for this different response to the two secretogogues is not clear. However, Mock *et al.* (9) demonstrated that the time required for internalization of one-half of bound ^{125}I-oLH from the membrane of ovine luteal cells was 0.4 ± 0.18 hr while 22.8 ± 2.3 hr was required for internalization of one-half of the membrane-bound ^{125}I-hCG. Thus, hCG receptor complexes may remain on the cell surface and continue to stimulate progesterone secretion. It has also been suggested (3, 10) that receptors bind a single molecule of hormone after which the hormone–receptor complex is internalized and the hormone degraded. This appears to be a mechanism for deactivating the hormone–receptor complex and for recycling of the receptor. If this is the case, the decline in progesterone secretion after a 15-min pulse of oLH may be a consequence of rapid internalization of receptor–hormone complex. The fact that this decline is observed even when oLH is continuously present in the medium suggests that either the number of available receptors decreases to a point below which steroidogenesis can be stimulated (down-regulation of receptors) or that receptors have been uncoupled from adenylate cyclase (desensitization). However, data on the number of occupied vs unoccupied and total receptors as well as hormone stimulated cyclase activity

would be needed to confirm these speculations.

We conclude that the steroidogenic responses of ovine small luteal cells to oLH and hCG are different. We suggest this difference may be related to differences in hormone stimulated receptor internalization.

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