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# Prolactin, Testicular Growth and LH Receptors in the Ram Following Light and 2-Br-α-Ergocryptine (CB-154) Treatments

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## ABSTRACT

The influence of prolactin (PRL) on testicular growth and luteinizing hormone receptors (LH-R) in the ram has been investigated. Unoccupied LH-R were estimated in the 5000 × g testicular membrane fraction. The specific activity of the [$^{125}$I]-oLH (enzymatically radioiodinated) was 20–40 μCi/μg and maximum binding activity reached 15–25%. Kinetic curves indicated that incubation for 20 h at 20°C was the optimum condition to obtain the maximum specific binding activity. Enzymatic treatment has demonstrated the lipoprotein structure of the LH-R. The specificity of the assay was confined to LH or human chorionic gonadotropin molecules. The equilibrium association constant (Ka) of the LH/receptor interaction was $10^{10}$ $M^{-1}$ and the number of LH-R determined using Scatchard analysis was ∿5 fmoles/mg membrane protein. The precision of the assay was 18%.

Plasma prolactin levels decreased by CB-154 treatment or increased by photostimulation did not change either the LH-R number or the equilibrium association constant. However, in the two experiments, a delay in the beginning of testicular growth was observed in rams treated with CB-154. In contrast to what has been found in rodents, these results suggest that, in the case of seasonal variations, high plasma prolactin levels influence changes in testicular activity without any involvement of the speculated regulation by prolactin of the LH-R number.

## INTRODUCTION

A relationship between plasma prolactin (PRL) concentrations, the number of testicular luteinizing hormone receptors (LH-R) and the testicular weight has been demonstrated in the hamster (Bex and Bartke, 1977). This suggests a possible synergism between luteinizing hormone (LH) and PRL in their action on the growth and/or activity of the testis (Bartke and Dalterio, 1976) in animals such as the hamster, where sexual activity is influenced by the photoperiod.

In the ram, where sexual activity undergoes marked seasonal variation, it is known that the beginning of testicular growth, which occurs in July, is preceded by activation of the hypothalamo-hypophyseal axis which successively affects PRL (Ravault, 1976), luteinizing hormone-releasing hormone and LH (Pelletier, 1971). Thus, it was concluded that PRL may play a role in testicular growth in the ram and, in particular, may regulate the number of LH-R. To test this hypothesis, hypoprolactinemia was induced by bromocryptine (CB-154) administration to rams under two different photoperiodic situations.

The quantitation of the number of LH-R in the ram testis necessitated a methodological study which proved difficult because the proportion of interstitial tissue in the ram is less than in other species such as the boar. In addition, unlike in the rat, the Leydig cells could not be isolated because of the large amount of connective tissue in the intertubular spaces. To show that the physiological results could not be explained by defective methodology, the validation of the assay method of the LH-R in the testis of the ram has been extensively investigated.

## MATERIALS AND METHODS

### *Materials*

Ovine LH (oLH-M3, 1.8 NIH-LH-S1) and ovine follicle stimulating hormone (oFSH-M3, 20.5 NIH-FSH-S3) were provided by Dr. Jutisz (CNRS-Gif/yvette, France). Purified bovine thyrotropin (NIH-TSH-B2) and ovine PRL (NIH-P-S6) were obtained from NIH, Bethesda, MD; human chorionic gonadotropin (hCG) from Diosynth, Paris, France and synthetic adreno-

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corticotropic hormone (ACTH-Synactene) from CIBA-Geigy, Rueil M., France. Lactoperoxidase, glucose oxidase, β-D⁺-glucose, phospholipases $A_2$, C and D, neuraminidase, trypsin, α-chymotrypsin, collagenase, bovine serum albumin (BSA, RIA grade) and phenylmethyl-sulphonyl-fluoride (PMSF) were purchased from Sigma, St. Louis, MO; Deoxyribonuclease I (DNase I) and ribonuclease A (RNase A) from Worthington, Freehold, NJ; 2-Br-α-ergocryptin (CB-154) from Sandoz Ltd., Basle, Switzerland. Sephadex was purchased from Pharmacia Uppsala, Sweden and carrier-free $^{125}$I-Na (IMS 300), 1,2,6,7-[$^3$H]-testosterone (81 Ci/mmole) from Amersham, Buckingamshire, UK; testosterone from Steraloids, Pawling, NY.

*Radioiodination Procedure*

Ovine LH was enzymatically radioiodinated at room temperature in the presence of lactoperoxidase, glucose oxidase and β-D⁺-glucose as described by Tower et al. (1977). The radioactive oLH was purified by successive elution on Sephadex columns [P10 (G25) followed by K30 (G100)] previously equilibrated with 25 mM Tris HCl buffer (pH 7.6) containing 0.1% (w/v) BSA. After elution on the G25 Sephadex column, 60% of the added radioactivity was found in the protein fractions. Eighty to 90% of the radioactivity in the G100 Sephadex fraction precipitated with an excess of specific anti-LH antibody. The specific activity of the [$^{125}$I]-oLH (20–40 μCi/μg) determined either by trichloracetic acid precipitation (Shiu and Friesen, 1974) or autocompetition curves (Fig. 1) (Ketelslegers et al., 1975) was the same. When the LH was radioiodinated by the chloramine T method (Greenwood et al., 1963), the specific activity of the [$^{125}$I]-oLH was higher, but the maximum binding activity to testicular membranes was decreased by 70% compared with the [$^{125}$I]-oLH iodinated by the glucose oxidase method.

*Preparation of Testicular Membranes*

Ram testes were recovered either at slaughter or castration. They were immediately dissected and weighed (without the albuginea) and where applicable stored in liquid nitrogen. The assay for the LH-R was always carried out on fresh testis material. All the following steps were carried out at 4°C, in 25 mM Tris HCl buffer, pH 7.6, containing 10 mM $MgCl_2$ (TH buffer). The testis was homogenized in a Waring blender (0.1 g wet weight/ml), followed by 3 strokes in a Potter homogenizer (Dounce). The homogenate was centrifuged for 30 min at 120 × g, the pellet was discarded and the supernatant centrifuged for 30 min at 10,000 × g. Then the pellet was resuspended in TH buffer containing 1 mM PMSF. This was left for 24 h at 4°C to desaturate the binding sites occupied by endogenous LH, as suggested by Esfahani et al. (1976). (We verified that this method of "desaturation" allowed 20% more sites to be assayed.) After 24 h, the membrane suspension was centrifuged for 30 min at 5,000 × g and the pellet resuspended at a concentration of 1 g wet weight/ml. The concentration of membrane proteins was then measured by the method of Lowry et al. (1951) (taking BSA as reference) after hydrolysis of the membranes with 0.1 N NaOH for 1 h at 65°C.

*Assay Procedure*

All incubations were carried out in triplicate. Plasma membrane aliquots equivalent to 0.5 mg protein were incubated at 20°C for 24 h with [$^{125}$I]-oLH (20,000 cpm) in 500 μl TH buffer containing 0.1% (w/v) BSA. Similar incubations were performed with an excess of nonradioactive oLH to determine nonspecific binding (NSB) or with increasing amounts of nonradioactive oLH for competitive inhibition used for Scatchard analysis. Figure 6B shows that 250 ng LH were sufficient to determine NSB since the binding sites were saturated with only 10 ng LH. Furthermore, an equivalent NSB was found when an excess of 1000 ng LH was added. After incubation, 1 ml cold TH buffer was added. LH bound to binding sites was separated from free hormone by centrifugation (15 min at 5000 × g). The pellets were washed with an additional 2 ml TH buffer to decrease NSB (Catt et al., 1972), recentrifuged and counted in a Packard Auto Gamma counter. Specific binding (SB) was defined as the difference between the total binding (TB) (incubation without excess oLH) and NSB.

*Characteristics of Ovine Testicular LH Receptor Assay*

*Kinetics.* The binding of [$^{125}$I]-oLH to the testicular membranes has been shown to be a time and temperature dependent process (Fig. 2). At 34°C (internal temperature of ram testes from Waites and

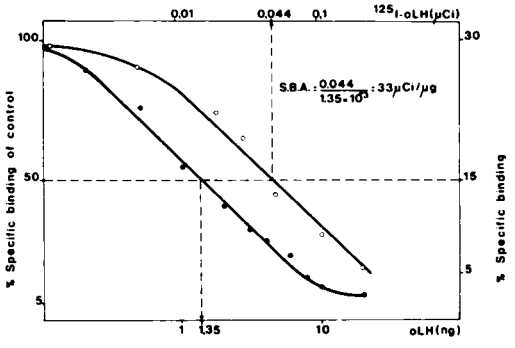


FIG. 1. Determination of specific binding activity by autocompetition of [$^{125}$I]-oLH on ovine testicular membranes. Curve A (○): Saturation curve of testicular LH receptors (0.5 mg protein/tube) with increasing amounts of [$^{125}$I]-oLH. NSB was determined by adding 250 ng oLH (see top abscissa and right ordinate). Curve B (●): Competitive inhibition curve with increasing amounts of unlabeled oLH added to [$^{125}$I]-oLH (20,000 cpm) and 0.5 mg membrane protein/tube (see lower abscissa and left ordinate). Specific binding activity (SBA) was calculated when the quantity of [$^{125}$I]-oLH at 50% maximum specific binding (Curve A) was divided by the quantity of unlabeled oLH used to obtain 50% displacement of buffer control (curve B).

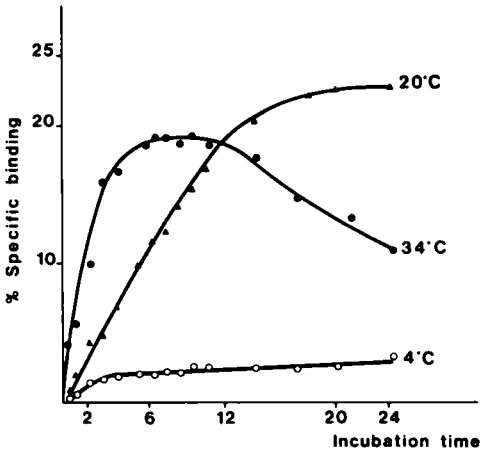


FIG. 2. Effect of time and temperature on the binding of [$^{125}$I]-oLH to ovine testicular membranes. Procedures for determining specific binding are described in Assay Procedure (Materials and Methods). Values are expressed as percentage of [$^{125}$I]-oLH specifically bound as a function of time. Incubation at different temperatures: (●) 34°C (▲) 20°C; (○) 4°C.

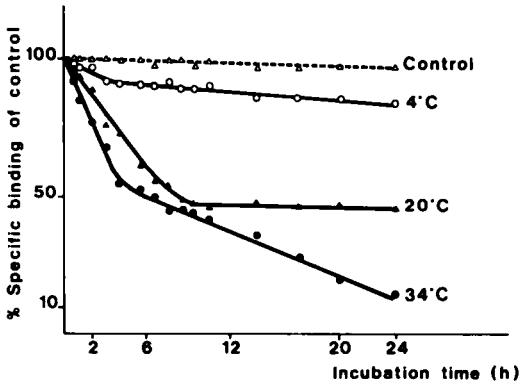


FIG. 3. Effect of time and temperature on the dissociation of LH receptor complex. Membranes and [$^{125}$I]-oLH (20,000 cpm) were incubated 20 h at 20°C in the presence or absence of 250 ng oLH/tube. Specifically bound LH thus determined was taken as 100% bound value. Another series of tubes incubated in the same way (without unlabeled hormone) was divided into 4 sets. At zero time for 3 sets, 250 ng unlabeled oLH was added to each tube. Each set was incubated at (●) 34°C, (▲) 20°C or (○) 4°C. The fourth set (△) was incubated at 20°C without unlabeled oLH and was taken as control.

Moule, 1961) most of the SB was obtained within 7 h but the degradations of the binding sites and/or the hormone occurred rapidly and NSB was elevated. Maximal hormone binding was obtained within 20 h at 20°C and the binding was weak at 4°C. The dissociation of the LH-R complex was also found to be dependent on time and temperature (Fig. 3). At 34°C, the dissociation occurred more rapidly than at 20°C and reached 50% within 6 h. A further decrease of SB could be due in part to binding sites and/or hormone degradations. At 20°C and without excess of nonradioactive LH, the hormone receptor complex remained stable for 24 h.

*Effect of protein concentration.* The SB of [$^{125}$I]-oLH to testicular membranes increased linearly up to 1 mg membrane protein/tube (Fig. 4). The relationship was then nonlinear until saturation. Maximum binding activity (15–25%) was reached with 4 mg membrane protein/tube. In contrast, the NSB showed a linear relationship with the concentration of membrane protein.

*Specificity.* Nonradioactive oLH or hCG inhibited the binding of [$^{125}$I]-oLH to ovine testicular membranes in a dose-dependent manner (Fig. 5). There was no interference of oPRL and ACTH, while oFSH and bTSH inhibited the binding of [$^{125}$I]-oLH to the membranes only at 100 times the concentration of oLH, compatible with LH contamination. The binding of [$^{125}$I]-oLH to denaturated membranes by warming (1 h at 65°C) was similar to the NSB (Fig. 5).

*Effect of enzymes on binding to testicular membranes.* DNase I, RNase A, neuraminidase and phospholipase D had no effect on LH binding sites. Phospholipases $A_2$ and C both decreased SB and increased NSB. Proteolytic enzymes (collagenase, trypsin and α-chymotrypsin) reduced the binding sites for LH by a much greater percentage than the membrane protein content (Table 1).

*Binding constant and determination of LH receptor sites.* Hormone receptor binding was assumed to be a second order reaction. Scatchard analysis (Scatchard, 1949) of the equilibrium data obtained from competitive inhibition curves was used to estimate the concentration of binding sites and the equilibrium association constant (Ka). A molecular weight of 38,000 (Gospodarowicz, 1972) was used to calculate the molar concentration of LH bound. The LH binding to the membranes was a saturable phenomenon. With increasing amounts of either [$^{125}$I]-oLH (Fig. 6A) or nonradioactive oLH (Fig. 6B)

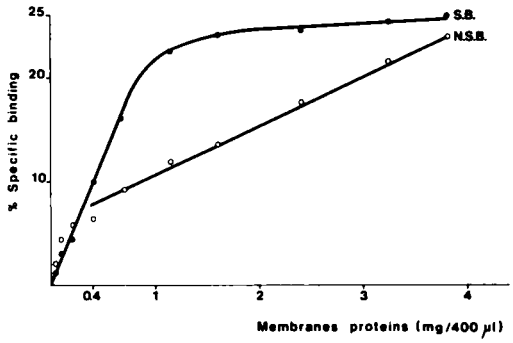


FIG. 4. Effect of membrane protein content on specific binding of [$^{125}$I]-oLH. Incubation, determination of specific binding and protein determination are described in Materials and Methods. (●) Specific binding (SB); (○) Nonspecific binding (NSB).

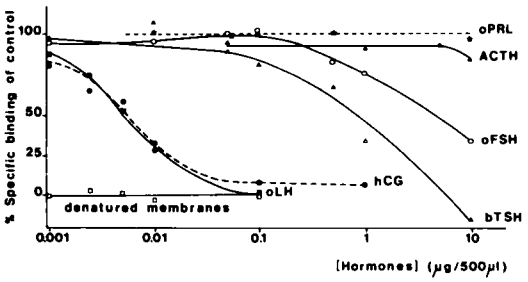


FIG. 5. Specificity of LH receptor interaction. Displacement of [$^{125}$I]-oLH bound to ovine testicular membranes with increasing amounts of different hormones added. (□) Membranes first heated at 65°C for 1 h and incubated with increasing amounts of oLH.

saturation was obtained with 10 ng LH when incubated with 0.5 mg membrane proteins. Scatchard analysis of data obtained from these two curves (Fig. 7) gave comparable estimates of the equilibrium association constants (Ka = 2.15 and 1.83 $10^{10}$ $M^{-1}$, respectively) and equivalent number of LH binding sites (n = 4.86 and 5.02 fmoles/mg protein, respectively).

*Precision of the method.* Testicular membranes equivalent to 0.20, 0.32 and 0.80 mg protein were incubated. No difference in the number of LH binding sites (expressed per mg protein) was observed (Fig. 8). The Ka was not significantly different but seemed to decrease when the amounts of protein used approached the limit of proportionality between percentage SB and concentration of membrane proteins incubated (Figs. 4, 8). The reproducibility of the estimation of the binding sites numbers and Ka was ±18% in subsequent assays (n = 6) using the same amount of protein provided from the same testicular membrane preparation.

*Hormone assays.* Prolactin (Kann, 1971) and LH (Pelletier et al., 1968) were measured by radioimmunoassay. To measure the intratesticular testosterone, 2 g of testis taken from the center of the gonad were homogenized in 10 ml water. The homogenate was incubated for 30 min at room temperature with 2000 cpm [$^3$H]-testosterone to estimate the extraction yield of testosterone. Steroids were extracted with 5 volumes of cyclohexane-ethyl acetate (1:1, v/v). Testosterone was separated from other steroids by celite chromatography and assayed as described by Garnier et al. (1978).

*Physiological protocols.* Experiment I: Six 2-year-old Romanov rams, submitted to natural seasonal light variation, were divided into two equal subgroups: on subgroup was injected s.c. twice a day with 2 mg CB-154 dissolved in 1 ml ethanol:0.9% NaCl (60:40, v/v) for 50 days from June 14 to August 4. The other subgroup (controls) received solvent only under the same conditions.

Experiment II: Ten 2-year-old Préalpes-du-Sud rams were submitted to artificial light treatment in a lightproof building. Light was given for 7 h (0800–1500 h) and in addition a light pulse of 1 h was placed 16–17 h after "dawn"; this treatment has been shown to stimulate PRL release (Ravault and Ortavant, 1977). Animals were divided into two equal subgroups; one received the CB-154 treatment as described in experiment I, the other group acted as control. Light treatment started on December 28, CB-154 treatment on January 8 and both light and CB-154 treatments were stopped 43 days later on February 20.

In both experiments the rams were castrated at the end of treatments. Testes were rapidly weighed, then homogenized at 4°C as described above. Jugular vein blood samples were taken once a week during the experiments at 0900 h. Plasma samples were stored at −15°C until assessment of plasma hormone levels. Testicular weight changes were estimated after measuring testicular diameter with a caliper-square (the

TABLE 1. Effect of enzymatic treatments.[a]

| Enzymes | Ratio (µg/ml) | % SB | % NSB | % Protein |
|---|---|---|---|---|
| Controls | – | 100 | 100 | 100 |
| Deoxyribonuclease I | 40 | 98.7 | 85.7 | 103.4 |
| Ribonuclease A | 40 | 93.4 | 77.2 | 96.2 |
| Neuraminidase | 40 | 96.0 | 93.8 | 100.4 |
| Phospholipase $A_2$ | 10 | 9.7 | 276.0 | 99.2 |
| Phospholipase C | 10 | 29.1 | 212.0 | 102.1 |
| Phospholipase D | 40 | 99.2 | 103.6 | 100.4 |
| Collagenase | 100 | 28.2 | 115.2 | 81.9 |
| Trypsin | 40 | 1.3 | 116.0 | 65.1 |
| α-Chymotrypsin | 20 | 27.3 | 116.4 | 76.5 |

[a]Testicular membranes were incubated for 30 min at 37°C in 3 ml Tris HCl buffer with enzymes in the amounts indicated (10 mM $CaCl_2$ were added to phospholipase $A_2$, C and D). At the end of incubation time, 10 mM PMSF were added to stop proteolytic enzyme activity and the pellets were washed 3 times with 30 ml Tris HCl buffer and centrifuged 30 min at 5000 X g. Pellets were resuspended in 3 ml buffer and incubated for 20 h at 20°C. Results of 3 experiments are expressed as percentage of control values (incubated in the same conditions but without enzymes).

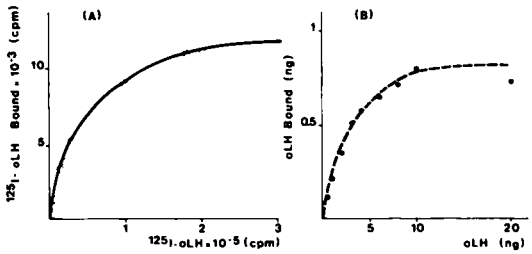


FIG. 6. Specific binding of oLH to ovine testicular membranes as a function of hormone concentrations. Membrane proteins (0.5 mg) incubated as described in Materials and Methods: A) with increasing amounts of [$^{125}$I]-oLH in the presence or absence of 250 ng unlabeled oLH; B) with increasing amounts of unlabeled oLH and constant amount of [$^{125}$I]-oLH (20,000 cpm).

correlation coefficient between testicular weight and diameter was 0.93).

*Statistics.* Results were expressed as mean ± SEM and statistical analyses were performed using Student's t test.

## RESULTS

### *Prolactin Levels and Testicular Growth*

*Experiment I.* The mean weekly plasma PRL levels in control rams varied between 100–300 ng/ml but, in the CB-154 treated animals, PRL concentrations fell significantly ($P<0.001$) after the first week to less than 5 ng/ml at the end of the treatment (Fig. 9A). Testicular weight increased significantly ($P<0.02$) between the beginning and the end of the experiment for both subgroups, but no difference was observed between control and treated rams at the end of the experiment (Table 2). Meanwhile testicular growth was delayed by 2 weeks in CB-154 treated rams; a significant difference ($P<0.05$) was observed during Weeks 3, 4 and 5 of treatment between control and CB-154 treated animals. Later, the testicular growth of CB-154 treated rams was equal to that of controls (Fig. 9B).

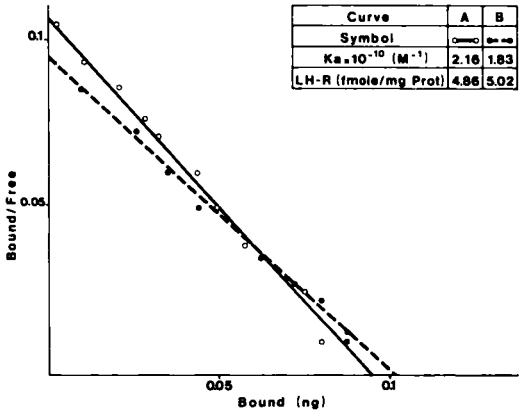


FIG. 7. Determination of affinity constant and number of LH receptor sites. Scatchard plot of data obtained from Figs. 6A,B. The slope of the line yields -1/Kd and the intercept on the abscissa yields the total oLH binding capacity, in ng, for 0.5 mg membrane protein. Radioiodination yields and specific activity were used to express [$^{125}$I]-oLH in ng which was then calculated in fmoles using molecular weight of 38,000.

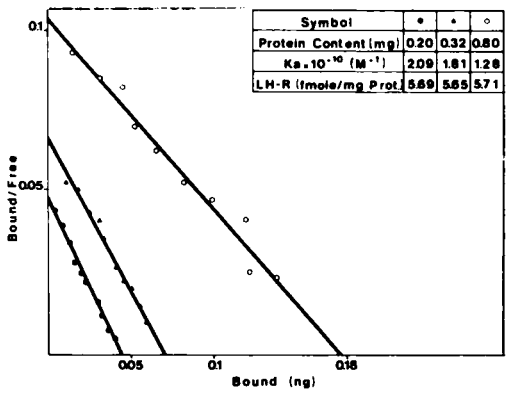


FIG. 8. Effect of membrane protein content on the estimation of number of LH receptor sites and affinity constant. Three concentrations of membrane proteins (0.20, 0.32, 0.80 mg) were incubated as described in Fig. 6B and were subjected to Scatchard analysis.

*Experiment II.* The plasma PRL level in control rams was 33 ± 7 ng/ml at the beginning of the experiment, after which it increased regularly and was higher than 100 ng/ml after 40 days of light treatment. In CB-154 treated animals, the plasma PRL levels were 24 ± 3 ng/ml before treatment, fell to 4–5 ng/ml 3 days after the first injection and remained low until the end of the experiment (Fig. 10).

Testicular weight was significantly increased ($P<0.05$) between the beginning and the end of the experiment in the control subgroup but not in CB-154 treated rams. However, the difference was not significant between control and CB-154 treated rams at the end of the experiment (Table 2).

### *Testicular LH Receptors and Intratesticular Testosterone*

In both experiments, Ka values were comparable in the control and CB-154 treated rams

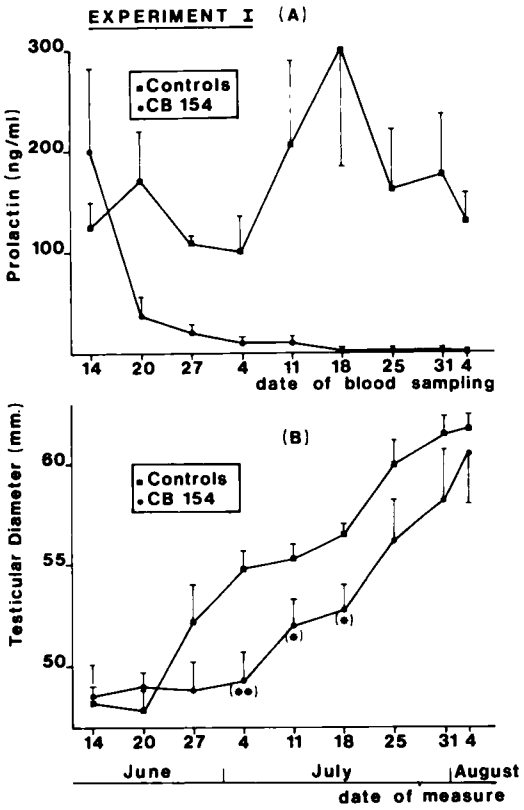


FIG. 9. Effect of 2-Br-α-ergocryptine treatment on A) plasma prolactin level and B) testicular diameter in rams under natural light variations (Experiment I, see Materials and Methods). (■) controls; (●) CB-154 treated rams. Mean ± SEM. Significant difference from controls: $^{(**)}P<0.01$; $^{(*)}P<0.05$).

and close to $1 \times 10^{10}$ $M^{-1}$. The overall mean LH-R number expressed as fmole/mg membrane proteins was significantly greater ($P<0.05$) in experiment I than in experiment II. However in neither case did the LH-R number per mg protein differ between the CB-154 treated and the control rams. Thus, the membrane protein content per g wet weight was equivalent for each subgroup of the two experiments. The intratesticular testosterone concentration did not differ significantly but had a tendency to decrease in each CB-154 treated subgroup of the two experiments (Table 2). Further, in experiment II, for the two subgroups at the time of castration, plasma LH levels were 3.6 ± 0.4 ng/ml and 2.9 ± 0.2 ng/ml (mean of 5 samples collected every 15 min for 60 min before surgery), respectively, for control and CB-154 treated rams and were not statistically different.

## DISCUSSION

The radioiodination procedure using glucose oxidase does not affect the LH binding ability because the same specific activity of radioactive LH was found by two different methods: the trichloracetic acid precipitation method, which takes the total radioiodinated hormone into account, and the autocompetition method, which involves only the [$^{125}$I]-oLH that has kept its binding ability. By comparison, the radioiodination procedure using chloramine T decreased the binding ability of the radioactive LH. Furthermore, this shows that radioiodination of the lactoperoxidase and the glucose oxidase used was negligible.

Binding was shown to be time and temperature dependent: the association of the radioactive LH to ovine testicular membranes was weak at 4°C as with the human testis (Hsu et al., 1978). At 20°C and 34°C, both association and dissociation time curves of LH receptor complex are comparable with binding of LH to human and with binding of FSH to bovine testicular membranes (Cheng, 1975). It is noteworthy that in the above experiments, SB of LH decreases after incubation for 7 h at 34°C as does SB of FSH to bovine testicular membranes. However, the stability of the LH receptor complex at 20°C justifies the incubation conditions used (20 h at 20°C) since SB is then maximal. LH receptor binding is specific because binding is inhibited only by hCG-like hormones and is destroyed by denatured target tissue. Binding capacity is lost after treatment with proteolytic enzymes and phospholipases $A_2$ and C, showing that LH binding sites are lipoprotein molecules. Increased NSB by phospholipases $A_2$ and C indicate that the structural integrity of the membranes must be retained.

LH receptor binding is characterized by a high affinity and a low saturation rate. The Ka of $1-2.10^{10}$ $M^{-1}$ reported in the present study is consistent with values reported for the rat testis (Catt et al., 1972) and more recently for the ovine corpus luteum (Diekman et al., 1978). The Ka is independent of membrane protein content up to 1 mg/tube. Scatchard analysis obtained from either [$^{125}$I]-oLH saturation curves or nonradioactive LH competitive inhibition curves allows comparable estimations of Ka and LH binding site numbers to be made with acceptable precision. A single class of binding sites has been found, whereas a second slope can be observed (not shown) when

TABLE 2. Effect of 2 Br-α-ergocryptine treatment on membrane protein content, testicular weight, LH receptors, affinity constant (Ka) and intratesticular testosterone.[a]

| | Experiment I | | | | Experiment II | | |
|---|---|---|---|---|---|---|---|
| | Controls | (A) | CB-154 Treated | (B) | Controls | (A) | CB-154 Treated |
| n Animals | 3 | | 3 | | 5 | | 5 |
| Membrane proteins (mg/g wet weight) | 8.3 ± 1.3 | NS* | 7.3 ± 1.9 | NS | 10.0 ± 0.8 | NS | 8.8 ± 0.6 |
| Testicular weight (g) | | | | | | | |
| Start | 100 ± 13 | NS | 109 ± 3 | NS | 124 ± 6 | NS | 133 ± 15 |
| (C) | P<0.02 | | P<0.02 | | P<0.05 | | NS |
| End | 180 ± 12 | NS | 169 ± 14 | P<0.05 | 147 ± 5 | NS | 141 ± 7 |
| LH receptors (fmole/mg protein) | 8.0 ± 0.7 | NS | 9.3 ± 0.6 | P<0.02 | 4.9 ± 1.5 | NS | 4.9 ± 1.7 |
| Ka X $10^{-10}$ $M^{-1}$ | 1.2 ± 0.1 | NS | 1.2 ± 0.2 | NS | 1.1 ± 1.7 | NS | 0.9 ± 0.8 |
| Intratesticular testosterone (ng/g wet weight) | 26.3 ± 7.0 | NS | 15.9 ± 4.3 | NS | 17.5 ± 7.2 | NS | 12.8 ± 3.7 |

[a]Mean values ± SEM in rams with two different induced PRL levels (Experiments I and II, see Materials and Methods).
(A) Difference between controls and CB-154 treated rams.
(B) Difference between Experiments I and II (the 2 subgroups were pooled).
(C) Difference between start and end of experiments.

*NS, difference not significant.

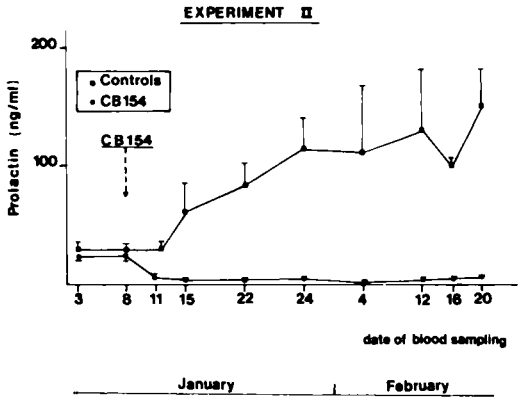


FIG. 10. Effect of 2-Br-α-ergocryptine treatment on plasma prolactin level in rams subjected to an artificial light regimen stimulatory for PRL release (Experiment II; see Materials and Methods). (■) controls; (●) CB-154 treated rams. Mean ± SEM.

testes stored in liquid nitrogen are used as shown for angiotensin II receptors (Glossmann et al., 1974). The ovine testicular LH-R concentration (5 fmoles/mg protein) is less than that reported for the ovine corpus luteum (Diekman et al., 1978) where the proportion of LH target cells is greater than for the testis. A similar low LH-R number has been reported for the human testis (Davies et al., 1979; Hsu et al., 1978).

The physiological part of this study was designed to demonstrate the effect of PRL on the number of LH-R and on testicular growth. This effect has been clearly shown in the hamster (Bex and Bartke, 1977). To do this, two distinct experiments were carried out. Experiment I began at a time when 1) the plasma level of PRL resulting from natural seasonal variations was at a maximum (Ravault, 1976) and when 2) the rapid phase of testicular growth had not yet begun (Pelletier, 1971). Under these conditions, a decrease in PRL due to CB-154 treatment did not affect the number of LH-R. In addition, at the end of the experiment, no difference in testicular weight could be found between control animals and those treated with CB-154. This confirms the results found previously in the lamb by Ravault et al. (1977). Nevertheless, it appears that there is a transitory effect of hypoprolactinemia in rams treated with CB-154, because a delay of 2 weeks in the beginning of testicular growth was observed. It is possible that experimentally-induced hypoprolactinemia was not sufficient to reduce the number of LH-R because the latter is perhaps determined by the PRL level which is elevated in the ram from the month of April onward (Ravault, 1976).

The second experiment was carried out in December when plasma PRL levels are normally low (Ravault, 1976). To increase the difference between control rams and those treated with CB-154, the plasma PRL level was increased in control animals by a light regimen which included a flash during the dark phase (Ravault and Ortavant, 1977), while this increase was inhibited by the drug in animals which received CB-154. Thus, the prolactin levels in CB-154 treated rams were markedly below those of normal animals during the nonbreeding season, while those of photostimulated controls approached the PRL level of rams during the breeding season. Under these conditions, the beginning of testicular growth was observed in control rams, but not in treated rams, and no difference in the number of LH-R was observed. The absence of a pulse of plasma LH immediately before castration does not allow one to invoke a phenomenon such as down-regulation (Sharpe, 1976; Purvis et al., 1977; Huhtaniemi et al., 1978) to explain the finding that in the control rams, the number of LH-R was no greater than in CB-154 treated rams. It is not known whether the difference in LH-R numbers between experiments I and II is due to the different light regimens or to breed differences between the two groups of animals.

Thus, in neither of the experiments reported here is it possible to demonstrate a direct effect of prolactin on the regulation of ovine testicular LH-R. This result differs from results obtained by PRL injection in the male hamster (Bex and Bartke, 1977) and male mouse (Bohnet and Friesen, 1976), or by CB-154 injection in the male (Aragona et al., 1977) and female rat (Holt et al., 1976), where PRL was shown to have a direct "all or none" effect on the LH-R number. Furthermore, the CB-154-induced decrease in testicular weight that has been reported previously in the hamster (Bex et al., 1978) was not seen in the ram. Instead, a delay in the beginning of testicular growth was observed. It should be noted that a direct effect of PRL on the Leydig cells would imply the presence of membrane PRL binding sites. The existence of PRL binding sites has already been shown in the rodent testis (Aragona and Friesen, 1975; Barkey et al., 1977; Charreau et al., 1977). However, until now, no specific binding of PRL to highly purified testicular plasma

membranes (100,000 x g pellet) of the ram has been found (results not shown).

Nevertheless, one cannot conclude that there is no effect of PRL on the testicular LH-R number in the ram for two reasons: First, PRL could have a short-lived effect on the LH-R number which could be sufficient to initiate testicular growth in the ram. This idea is enhanced by the suggestive changes in intratesticular testosterone content (Table 2). In experiment I, this assumed effect of PRL could occur either before the beginning of the CB-154 treatment or when the testicular size of the two subgroups differed (Fig. 9). Second, PRL binding sites could exist in undetectable quantities in the ram testis. In this case, the LH-R number could be regulated by the residual level of PRL (<5 ng/ml) in the CB-154 treated rams, either alone or in synergy with other hormones. Indeed, growth hormone in the mouse (Bohnet and Friesen, 1976), rat (Zipf et al., 1978) and hamster (Bex et al., 1978) and FSH in the immature rat (Chen et al., 1977) act synergystically with PRL in the regulation of testicular LH-R. In any case, the plasma PRL level is never less than 10 ng/ml under physiological conditions in the ram (Ravault, 1976). Therefore, the actual role of the large seasonal peak of PRL in the activation of testicular function is unlikely to enhance the LH-R number.

In conclusion, no stimulatory effect of prolactin on numbers of LH binding sites has been demonstrated so far in the ram. However, an inhibition of prolactin release delayed testicular growth. This result, inconsistent with those previously reported in rodents, suggests that prolactin might act on testicular function in the ram by a different pathway than in rodents.

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