PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 473-475 (1976)

FSH Stimulation of Leydig Cell Function in the Hypophysectomized Immature Rat (39571)

YII-DER IDA CHEN, ANITA H. PAYNE,[1] AND ROBERT P. KELCH

Steroid Research Unit, Reproductive Endocrinology Program, Departments of Obstetrics and Gynecology, Biological Chemistry and Pediatrics, The University of Michigan, Ann Arbor, Michigan 48109

Mechanisms involved in the development of Leydig cell function during maturation of the rat testis are still unknown. Studies by Swerdloff *et al.* (1) and Odell *et al.* (2) suggest that follicle-stimulating hormone (FSH) is a major factor in the development of testicular responsiveness to luteinizing hormone (LH) stimulation. These authors reported that *in vivo* administration of FSH to immature rats induced testicular responsiveness to LH. When 21-day-old rats were hypophysectomized and 5 days later treated with FSH for 10, 25, or 30 days, plus LH during the last 5 days of therapy, an increase in testicular responsiveness to LH was observed. This increase in responsiveness, as determined by an increase in the weights of the prostate, was related to the duration of exposure to FSH. The mechanism by which FSH increased Leydig cell responsiveness has not been ascertained. The present study was undertaken to elucidate the mechanism of action of FSH on Leydig cell function of the immature hypophysectomized rat.

Materials and methods. Hypophysectomized male Sprague–Dawley rats were obtained from Hormone Assay, Ltd. They were hypophysectomized at Day 20. All treatments were started 5 days after hypophysectomy. Rats were injected sc twice daily with 20 μg of NIH-FSH-S11, biological potency 1.15 × NIH-FSH-S1 and less than 0.01 × NIH-LH-S1, or with 1 μg of NIH-LH-S19, biological potency 1 × NIH-LH-S1 and less than 0.05 × NIH-FSH-S1. Control rats were given saline or the amount of LH equivalent to that found in 20 μg of FSH (0.2 μg), twice daily. After 5 or 10 days of treatment, animals were killed and testes were removed and weighed. One testis was used to determine the amount of LH receptor and the other testis was used to measure testicular responsiveness to LH stimulation. Since it has been demonstrated previously that ^{125}I-hCG and ^{125}I-LH bind to the same receptor in a subcellular fraction of the rat testis (3), highly purified hCG (10,000 IU/mg) was labeled with ^{125}I by a chloramine-T technique as previously described (4) and used to quantitate testicular LH receptors. Testes were homogenized in 0.25 *M* sucrose buffered with 0.05 *M* HEPES, pH 7.4 (HS). The homogenate was centrifuged for 30 min at 20,000*g*. The 20,000*g* pellet was resuspended in 1 ml of HS. Aliquots of 0.1 ml were incubated in a total volume of 0.2 ml of HS containing 0.1% BSA, 5 m*M* $CaCl_2$ (HSB-Ca), and 100,000 cpm of ^{125}I-hCG (~60,000 cpm/ng), and 100-fold excess of radioinert hCG in tubes in which nonspecific binding was determined. Incubations were carried out at 34° for 1.5 hr. Reactions were stopped by addition of 1 ml of ice-cold HSB-Ca. Hormone-bound membrane fractions were collected by centrifugation at 20,000*g* for 30 min. The supernatant was removed and the 20,000*g* membrane fraction was washed two more times with cold HSB-Ca. Radioactivity in each tube was determined by counting for 1 min in an automatic gamma spectrometer. Specific binding was calculated as the difference between binding in the presence and absence of excess radioinert hCG and expressed as counts per minute per testis.

To measure the effect of LH on testicular responsiveness, one-half of the contralateral testis was incubated in 1 ml of Krebs–Ringer buffer containing 2 mg/ml of glucose and 20 ng/ml of NIAMDD-Rat LH-I-3 (biological potency of 1 × NIH-LH-S1 units/mg) and the other half of each testis was incubated in buffer without added rat LH. In a preliminary study, it was determined that 10 ng/ml

[1] Author to whom requests for reprints should be addressed.

of rat LH resulted in maximum testosterone production. Incubations were performed for 4 hr at 34° under 95% O_2–5% CO_2. To measure total testosterone, testes were homogenized in media; [^{3}H]testosterone was added to monitor for losses and steroids were extracted with 75% ethanol, followed by two extractions with absolute ethanol. The dried ethanol extract was dissolved in 1 ml of phosphate-buffered saline containing 0.1% pig gelatin (gel PBS). The gel PBS solution was extracted with 10 vol of benzene:hexane (1:2). Each extract was dried and reconstituted in 1 ml of gel PBS. Aliquots of 0.1 ml were taken for counting [^{3}H]testosterone and duplicate 50- and 200-μl aliquots were used for radioimmunoassay. Testosterone was measured by radioimmunoassay employing testosterone-11α-hemisuccinate-^{125}I-tyrosine methyl ester and a specific antiserum elicited in rabbits against testosterone-11α-hemisuccinate conjugated to BSA (5). Testicular responsiveness to LH is expressed as the difference in testosterone production with and without added LH.

Results. Changes in testicular weight, ^{125}I-hCG binding, and *in vitro* testicular responsiveness are presented in Fig. 1A, B, and C, respectively. Testes from the saline-injected hypophysectomized rats regressed during the 10-day period of the study as indicated by a decrease in testicular weight, a decrease in LH receptors per testis, and a decrease in testosterone production in response to LH. Administration of LH even at five times the amount found in the administered FSH did not prevent the loss in testicular weight or the loss in LH receptors per testis, but appeared to maintain the capacity for testosterone synthesis in spite of the decrease in number of LH receptors. In contrast, FSH administration significantly stimulated all three parameters and the stimulation seen with FSH appeared dependent on the duration of FSH treatment. Percent increase in testicular weight and in LH-binding capacity were similar. Therefore number of LH receptors expressed on a weight basis did not increase with FSH treatment. However, the percentage increase in testicular responsiveness to LH was markedly greater than the percentage increase observed for testicular weight or LH receptors. Basal tes-

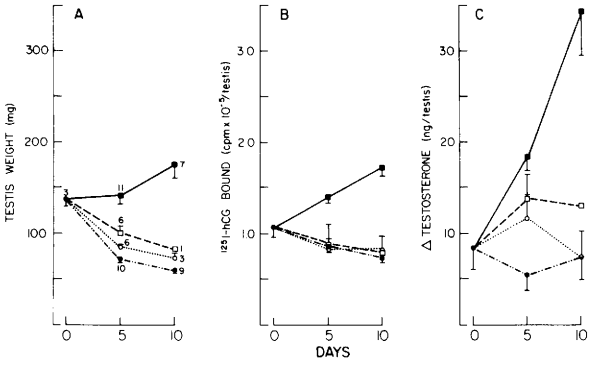

FIG. 1. Effects of hormone treatment on testes of hypophysectomized immature rats: saline (●), 0.4 μg of LH/day (○), 2 μg of LH/day (□), 40 μg of FSH/day (■). Each point represents mean ± SE. Numbers indicated at each point in (A) refer to all three graphs and represent number of rats. Student's *t* test was used to determine significant difference between means. (A) testicular weights in milligrams. $P < 0.001$ for all FSH-treated rats vs saline or 0.4-μg LH control rats. (B) Amount of ^{125}I-hCG bound in counts per minute per testis. Aliquots of resuspended 20,000*g* testicular membrane fractions were incubated in buffer containing 100,000 cpm of ^{125}I-hCG for 1.5 hr at 34°. For determination of specifically bound ^{125}I-hCG, nonspecific binding, as determined by incubation of ^{125}I-hCG in the presence of 100-fold excess radioinert hCG, was subtracted from total bound counts. $P < 0.001$ for all FSH-treated rats vs saline or 0.4-μg LH control rats. (C) *In vitro* testicular responsiveness to LH. One-half of each testis was incubated in 1 ml of buffer containing 20 ng/ml of rat LH and the other half of each testis was incubated in buffer without added rat LH. Incubations were performed for 4 hr at 34° under 95% O_2–5% CO_2. Total testosterone was measured by radioimmunoassay. $P < 0.001$ for FSH-treated rats vs saline controls; $P < 0.05$ for FSH-treated rats vs 0.4-μg LH controls at 5 days; $P < 0.005$ for FSH-treated rats vs 0.4-μg LH controls at 10 days.

tosterone production in testes from FSH- or LH-treated rats was the same as that observed in testes from saline-treated control rats. Thus, treatment with FSH increased testicular responsiveness per LH receptor. The ratios of LH-stimulated testosterone production (Δ testosterone in nanograms per testis) to receptor (cpm × 10^{-5} ^{125}I-hCG/testis) were 7.9, 13.2, and 20.0 at 0, 5, and 10 days, respectively.

Discussion. The present study demonstrates that FSH increased the number of LH receptors per testis. FSH was found to stimulate *in vitro* testicular testosterone production in response to LH. This LH-sensitive testicular response was enhanced to a greater extent than would be expected from the increase in the number of LH receptors after FSH treatment. These observations confirm and extend studies recently cited by Odell and Swerdloff (6). Thus, it seems unlikely that the change in testicular sensitivity to LH in the immature hypophysectomized rat can be attributed solely to increased numbers of LH receptors. The absence of a direct correlation between concentration of LH receptors and testicular response to LH has been observed by other investigators. Catt and Dufau (7) added increasing concentrations of hCG to Leydig cell preparations from adult testes, and observed maximal testosterone production when fewer than 1% of the LH receptors were occupied. They concluded that Leydig cells contain a large excess of LH receptors which are not necessary for eliciting maximal testosterone synthesis.

Previous studies by Means and Vaitukaitis (8) demonstrated that FSH binding was restricted to cells of seminiferous tubules. No binding of FSH to Leydig cells has been reported to date, suggesting that the effect of FSH on Leydig cell function may be mediated via seminiferous tubules. In preliminary studies, we did not observe any obvious differences in Leydig cell histology between hematoxylin- and eosin-stained sections of testes from saline- and FSH-treated rats. The exact mechanism by which FSH increased Leydig cell responsiveness to LH remains a matter of speculation. In this regard, we recently observed (5) that the *in vitro* testicular capacity to synthesize testosterone (measured as total testosterone production in the presence of LH) and the testicular 17β-hydroxysteroid dehydrogenase activity follow the same developmental pattern. This observation suggests that 17β-hydroxysteroid dehydrogenase may be a limiting factor in the ability of the testis to respond to LH stimulation and that this enzyme may be a key factor in the observed response to FSH treatment.

Summary. FSH treatment of hypophysectomized immature rats increased testicular weight, increased the number of LH receptors per testis, and increased the *in vitro* testicular testosterone production in response to LH. The increase observed in all three parameters was related to the duration of FSH treatment. The testicular response to LH was enhanced to a greater extent than the number of LH receptors. The specificity of the FSH effect was indicated by the observation that administration of LH had no effect on testicular weight or number of LH receptors. LH administration did not increase the *in vitro* testicular response to LH, but appeared to prevent the decrease in this response observed in saline-injected controls.

We thank Carol Craig for expert technical assistance. We also thank Dr. A. F. Parlow for the rat LH, NIAMDD-Rat LH-I-3, and NIAMDD for supplying the NIH-FSH-S11 and NIH-LH-S19. This study was supported by NIH Grant No. HD-08358.

1. Swerdloff, R. S., Walsh, P. C., Jacobs, H. S., and Odell, W. D., Endocrinology **88,** 120 (1971).
2. Odell, W. D., Swerdloff, R. S., Jacobs, H. S., and Hercox, M. A., Endocrinology **92,** 160 (1973).
3. Catt, K. J., Dufau, M. L., and Tsuruhara, T., J. Clin. Endocrinol. Metab. **34,** 123 (1972).
4. Tsuruhara, T., Van Hall, E. V., Dufau, M. L., and Catt, K. J., Endocrinology **91,** 463 (1972).
5. Payne, A. H., Kelch, R. P., Murono, E. P., and Kerlan, J. T., J. Endocrinol. (in press).
6. Odell, W. D., and Swerdloff, R. S., J. Steroid Biochem. **6,** 853 (1975).
7. Catt, K. J., and Dufau, M. L., Nature New Biol. **244,** 219 (1973).
8. Means, A. R., and Vaitukaitis, J., Endocrinology **90,** 39 (1972).

Received May 14, 1976. P.S.E.B.M. 1976, Vol. 153.