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DISSOCIATION OF LUTROPIN-INDUCED LOSS OF TESTICULAR LUTROPIN RECEPTORS AND LUTROPIN-INDUCED DESENSITIZATION OF TESTOSTERONE SYNTHESIS *

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Summary

The relationship between changes in testicular lutropin receptors, as measured by specific binding of ^{125}I-labeled human chorionic gonadotropin, and testosterone synthesis in response to lutropin (testicular responsiveness) was studied in intact and hypophysectomized rats. Administration of a single 200-μg dose of ovine lutropin to intact rats results at 3 days in a 58% decrease in lutropin receptors associated with a parallel decrease in testicular responsiveness. A single 30-μg dose of lutropin to intact rats resulted in a comparable decrease in lutropin receptors with a transient increase in testicular responsiveness. Rats receiving twice-daily injections of 15 μg lutropin for 10 days exhibited a 48% decrease in lutropin receptors by day 3 which persisted during the 10-day treatment period, but was accompanied by a progressive increase in testicular responsiveness to lutropin. Hypophysectomy resulted in an 80% loss of receptors and a 72% loss in responsiveness 7 days after surgery. Daily treatment with lutropin initiated immediately following surgery resulted in a further dose-dependent decrease in lutropin receptors and a dose-dependent increase in testicular responsiveness. Loss of lutropin receptors was not due to occupancy of the receptor by exogenous lutropin. These studies demonstrate a dissociation between the negative regulation of lutropin receptors and testicular responsiveness to lutropin. Furthermore, the studies in hypophysectomized rats indicate that lutropin is the only hormone essential for maintenance of steroidogenesis and that this is independent of lutropin receptor concentration.

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Introduction

Recent reports have indicated that luteinizing hormone (lutropin) or human chorionic gonadotropin (gonadotropin) induced decrease in testicular gonadotropin binding capacity is accompanied by a decrease in cyclic AMP and testicular responsiveness in response to further lutropin/gonadotropin stimulation [1,2]. These observations suggest that testicular concentration of lutropin receptors are regulated by lutropin and that this regulation determines testicular responsiveness. Hsueh et al. [1] reported that the decrease in testicular gonadotropin binding capacity after a single administration of 200 I.U. of gonadotropin was temporally associated with a fall in responsiveness to gonadotropin in vitro as measured by cyclic AMP and testosterone synthesis. Studies from our laboratory have shown that 6 days following hypophysectomy an 85% reduction in gonadotropin binding capacity occurred which was associated with a 60% loss in testicular responsiveness to lutropin stimulation [3]. Although there is a relationship between the fall in responsiveness and the loss of binding capacity, the temporal relationships of these changes are not tightly coupled. Hsueh et al. [1] demonstrated that after a single administration of gonadotropin, maximal testosterone response had returned to control values by 5 days even though lutropin receptors were still less than 10% and we observed [3] that 3 days following hypophysectomy, in vivo testicular responsiveness to lutropin was still normal while gonadotropin binding capacity had decreased by 70%. The present study was undertaken to test whether testosterone synthesis in response to lutropin is determined by lutropin receptor concentration. The results of this study demonstrate that changes in lutropin receptor concentration do not determine changes in steroidogenic response to lutropin.

Materials and Methods

Adult male Sprague-Dawley rats (80—90 days old) were obtained from Hormone Assay Laboratory, Inc. (8159 South Spaulding Ave., Chicago, Ill., 60652). Intact rats were killed by decapitation at 0, 1, 3, 7 or 10 days after the initiation of treatment. Hypophysectomy was performed under ether anesthesia by a transauricular approach [4]. Sham operations were performed by furrowing into the sphenoid bone without entering the sella turcica. Hypophysectomized or sham-operated animals were decapitated between 08.00 and 12.00 h on the morning of the 7th post-operative day. Testes, ventral prostate and seminal vesicles including the fluid were immediately dissected and weighed. Estimates of completeness of hypophysectomy were made by gross examination of the sella turcica in each animal. Animals showing evidence of residual pituitary tissue were excluded from the experimental group.

Hormones were administered subcutaneously either as a single dose or as twice-daily injections as indicated in the figure legends for 6.5 days. Hormone treatments in hypophysectomized rats were instituted within 6 h of surgery. NIH-LH-S20, biological potency 1.19 × NIH-LH-S1, <0.05 × NIH-FSH-S1, was used for lutropin treatments. All preparations were dissolved in isotonic saline.

Control animals received equal volumes of saline on the same injection schedule as treated groups.

To determine lutropin receptor concentration, the right testis was decapsulated, homogenized with a Teflon glass homogenizer in 6 ml of 0.25 M sucrose buffered with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES), pH 7.4. The homogenate was centrifuged at 20 000 × *g* for 30 min. The resulting pellet was resuspended in 2 ml of buffered 0.25 M sucrose and 0.1-ml aliquots containing approx. 250—350 μg protein were used to determine gonadotropin binding capacity. To remove bound lutropin from occupied receptors and determine total testicular gonadotropin binding capacity, half of the left testis was homogenized in 4 M $MgCl_2$ as previously described [5] with the following modification: after gentle homogenization in 4 M $MgCl_2$ the homogenate was diluted with buffered sucrose to 40 ml and thoroughly mixed prior to centrifugation. This method has been shown to remove lutropin without damage to the lutropin receptor [5]. The pellet was washed by vortex mixing with 20 ml of buffered sucrose and centrifuged again at 20 000 × *g*. This pellet was resuspended by homogenization in 4 ml buffered sucrose. The lutropin receptor assay was performed as previously described [3,5]. Specific hormone binding was calculated from the difference between binding in the presence and absence of excess unlabeled gonadotropin and expressed as pmol per testis on the basis of measured specific activities of the ^{125}I-labeled gonadotropin preparations.

Testicular responsiveness to lutropin was determined in some animals by measuring serum testosterone concentrations 2 h after the administration of 25 μg lutropin intraperitoneally. This amount of lutropin, administered 2 h before decapitation, had no measurable effect on gonadotropin binding capacity in either intact or hypophysectomized rats. Trunk blood was collected after decapitation, and serum testosterone was measured by a modified radioimmunoassay [3,6]. Student's impaired *t*-test was used for statistical comparisons.

Results

A single 200-μg injection of lutropin to intact adult rats resulted in a 58% fall in lutropin receptor concentration at 3 days, followed by a gradual increase to 75% of control values at 7 days. The majority of the decrease in lutropin receptors at 3 days was due to "loss" of receptors and not due to occupancy by lutropin (Fig. 1A) since removal of bound lutropin by $MgCl_2$ treatment did not restore binding capacity to control values. The loss of lutropin receptors was accompanied by a parallel 50% decrease in testicular responsiveness. However, responsiveness to lutropin returned to greater than control values by 5 days (Fig. 1B) when binding capacity was still at 72% of control values.

Administration of a single 30 μg dose of lutropin caused a similar fall in lutropin receptor concentration as was seen with 200 μg lutropin. A progressive increase in lutropin receptor concentration was observed the next 4 days to 82% of control values (Fig. 2A). This dose of lutropin was associated with an increase in testicular responsiveness at day 3 in contrast to the fall seen with the higher dose (Fig. 2B).

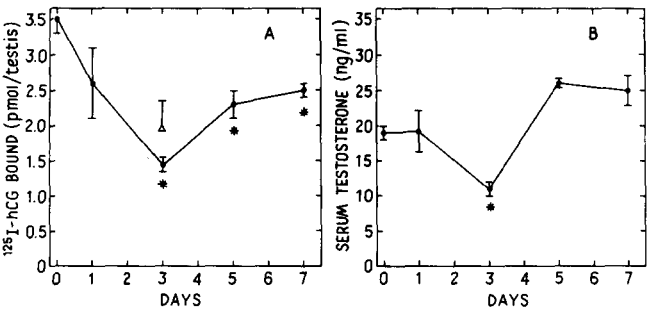

Fig. 1. Effect of a single dose of 200 μg lutropin on lutropin receptor concentration as measured by ^{125}I-labeled gonadotropin binding (A) and on testicular responsiveness to lutropin as measured by serum testosterone concentration (B) in intact adult rats. Each point represents mean ± S.E. of 12—21 rats. ^{125}I-labeled gonadotropin binding (total binding) to lutropin receptors of four contralateral testes treated with 4 M $MgCl_2$ (△). For details of experimental methods see text. * Significantly different from 0 day controls, $P < 0.01$.

Administration of 15 μg lutropin twice per day for 10 days to intact animals resulted in a 48% reduction in lutropin receptor concentration (Fig. 3B). This fall occurred by day 3 and did not change significantly during the remainder of the 10-day treatment period. The decrease in lutropin receptor concentration was due to a "loss" in receptor and was not a result of occupancy by lutropin (Fig. 3B). Despite this "loss" in gonadotropin binding capacity, testicular responsiveness to lutropin increased following the continuous twice-daily injections of 15 μg lutropin (Fig. 3A).

The effects of hypophysectomy and lutropin treatment on testicular lutropin receptor concentration and testicular responsiveness to lutropin are presented in Table I. 7 days post-hypophysectomy, lutropin receptor concentration was 20% and responsiveness to lutropin was 28% of that found in saline-injected control rats. Treatment with lutropin in doses of 5, 25 or 50 μg daily within 6 h of hypophysectomy resulted in a dose-dependent decrease in gonadotropin binding capacity for each dose level ($P < 0.05$) and a dose-dependent increase in testicular responsiveness to lutropin. At 50 μg/day a 90% loss in gonadotropin binding capacity was observed (mean ±S.E. = 0.36 ± 0.1 pmol/

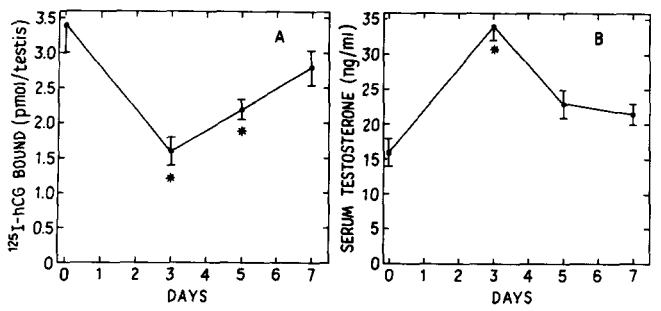

Fig. 2. Effect of a single dose of 30 μg lutropin on lutropin receptor concentration as measured by ^{125}I-labeled gonadotropin binding (A) and on testicular responsiveness to lutropin as measured by serum testosterone concentration (B) in intact adult rats. Each point represents mean ± S.E. of four rats. For details of experimental methods see text.

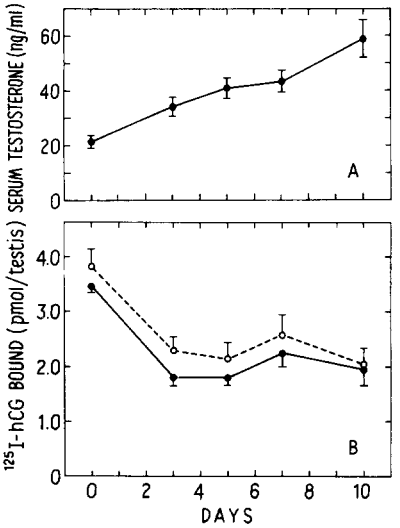

Fig. 3. Effect of twice-daily injections of lutropin (30 μg/day) on lutropin receptor concentration as measured by ^{125}I-labeled gonadotropin binding (B) and on testicular responsiveness to lutropin as measured by serum testosterone concentration (A). Each point represents mean ± S.E. of seven rats except for three rats on day 10. ^{125}I-labeled gonadotropin binding (total binding) to lutropin receptors of 4 M $MgCl_2$-treated contralateral testes (○- - - - -○). For details of experimental methods see text.

testis). The decrease in lutropin receptor concentration was not a result of occupancy since contralateral testes from these animals had similar gonadotropin binding capacities (0.39 ± 0.1 pmol/testis) after bound lutropin had been dissociated with 4 M $MgCl_2$.

The dose-dependent increases in testicular secretion of testosterone in response to lutropin was also reflected in the effects observed on the androgen-dependent organ weights (Table II). 7 days following hypophysectomy, ventral prostate and seminal vesicle weights had decreased to approx. 1/3 of controls.

TABLE I

EFFECT OF HYPOPHYSECTOMY AND LUTROPIN TREATMENT ON LUTROPIN RECEPTOR CONCENTRATION AS MEASURED BY ^{125}I-LABELED GONADOTROPIN BINDING AND TESTICULAR RESPONSIVENESS TO LUTROPIN IN ADULT MALE RATS

Rats (80—90 days) were hypophysectomized. Hormone treatments were initiated within 6 h after surgery and continued on a twice-daily schedule for 6.5 days. Animals were killed in the morning of day 7. For details of experimental methods see text.

Treatment group	No.	^{125}I-labeled binding (pmol/testis)	No.	Testicular responsiveness to lutropin (ng testosterone/ml serum)
Sham operation + saline	(70)	3.65 ± 0.11	(23)	24.7 ± 1.5
Hypophysectomy + saline	(38)	0.76 ± 0.06	(22)	6.3 ± 0.6
Hypophysectomy + lutropin (5 μg/day)	(20)	0.55 ± 0.05	(9)	17.5 ± 4.0
Hypophysectomy + lutropin (25 μg/day)	(10)	0.42 ± 0.03	(3)	55.5 ± 7.6
Hypophysectomy + lutropin (50 μg/day)	(10)	0.36 ± 0.07	(5)	57.0 ± 6.4

TABLE II

EFFECTS OF LUTROPIN ON WEIGHTS OF ANDROGEN-DEPENDENT ORGANS FROM HYPOPHYSECTOMIZED ADULT RATS

Rats (80—90 days) were hypophysectomized. Hormone treatments were initiated within 6 h after surgery and continued on a twice-daily schedule for 6.5 days. Immediately after decapitation organs were removed and weighed. Seminal vesicle weights include fluid.

Treatment group	No.	Ventral prostate (mg, mean ± S.E.)	Seminal vesicles (mg, mean ± S.E.)
Sham operation + saline	(35)	310 ± 20	899 ± 40
Hypophysectomy + saline	(44)	110 ± 10	252 ± 10
Hypophysectomy + lutropin (5 μg/day)	(20)	200 ± 20	442 ± 30
Hypophysectomy + lutropin (25 μg/day)	(10)	190 ± 20	554 ± 70
Hypophysectomy + lutropin (50 μg/day)	(10)	339 ± 50	993 ± 70

An increase in the weights of seminal vesicles and ventral prostate was observed in the hypophysectomized lutropin-treated rats (Table II).

Discussion

The present study demonstrates a marked dissociation between the negative regulation of lutropin/gonadotropin receptors and testicular responsiveness to lutropin, as determined by testosterone synthesis. Although all variations of treatment with lutropin in intact or in hypophysectomized rats results in a loss of lutropin receptors which was not due to occupancy, only a single 200 μg dose of lutropin to intact rats was accompanied by a decrease in testicular responsiveness in response to lutropin. The decrease in testicular responsiveness after a single large dose of lutropin cannot be attributed solely to the decrease in lutropin receptor concentration, since a single injection of 30 μg lutropin or twice daily injections of 15 μg of lutropin resulted in a similar loss of lutropin receptors, but was not accompanied by a loss in steroidogenic responsiveness. This observation suggests that a single large dose of lutropin has a negative effect on steroidogenesis by some mechanism other than decreasing lutropin receptor concentration. This hypothesis is supported in a recent study by Sharpe [2] who observed a loss in testicular lutropin receptors and in steroidogenic response after a single administration of 10 I.U. of gonadotropin to immature rats. He demonstrated that the decrease in steroidogenic capacity could not be attributed to "loss" of lutropin receptors since testes from gonadotropin-treated animals were unable to respond to dibutryl cyclic AMP any better than they did to lutropin/gonadotropin.

It has previously been reported that the absence of pituitary hormones results in the loss of lutropin receptors [3,7,8] and in a decrease in testicular responsiveness of lutropin [3]. In the present study we demonstrate that daily treatment with lutropin initiated within 6 h after hypophysectomy results in a dose-dependent increase in responsiveness despite a further decrease in lutropin receptors. The observed dose-dependent increase in responsiveness to lutropin

cannot be attributed solely to a release of stored testicular testosterone by the stimulatory intraperitoneal injection of lutropin, since the daily lutropin treatment resulted in the maintenance of the androgen-dependent organ weights. This indicates that significant amounts of testosterone were being synthesized and released each day of the treatment period.

Our results in the short-term, hypophysectomized adult rat indicate that lutropin is the only hormone essential for maintenance of testicular responsiveness regardless of lutropin receptor concentration. The data further indicate that the maintenance of lutropin receptors must be dependent directly or indirectly on the presence of other pituitary hormones.

Previous reports [1,2] on gonadotropin-induced negative regulation of lutropin receptors accompanied by a loss in steroidogenic response to lutropin were all based on studies employing a single administration of a large dose of either lutropin or gonadotropin. The present study demonstrates that this desensitization in testicular steroidogenesis occurs only after a single large non-physiological dose of lutropin; furthermore this decrease in steroidogenic capacity does not appear to be a result of a decrease in lutropin receptor concentration. Daily administration of lutropin both in the intact and in the hypophysectomized rat maintains or enhances steroidogenic capacity and exhibits no relationship to lutropin receptor concentration. Thus in these studies, the effect of lutropin on receptors appears to be distinct from the effect of lutropin on testicular responsiveness.

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