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In Vitro Studies of Prolactin Inhibition of Luteinizing Hormone Action on Leydig Cells of Rats and Mice (41973)

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Abstract. Previous *in vivo* studies have shown that in male rabbits prolactin inhibits the testosterone production stimulated by luteinizing hormone (LH) or human chorionic gonadotropin (hCG). This inhibition has now been studied *in vitro* using both mouse and rat testicular interstitial cells. First, the dose response of human LH (hLH) stimulation of testosterone was studied in detail using testicular interstitial cells from both species. Next, a small but stimulatory dose of hLH was selected and extensive prolactin doses were studied *in vitro*. NIH B-6 (bovine) prolactin in varying doses was added to the interstitial cells 30 min prior to the addition of a constant dose of hLH. Under these circumstances prolactin inhibited LH action over a wide range of doses. In both species a biphasic dose–response curve existed: large doses of 100 to 1000 ng/ml produced less inhibition or augmented LH action, compared to smaller doses. Next, entire hLH dose–response curves were produced in the presence of three doses of prolactin (0.33, 33, and 1000 ng/ml) as well as in the absence of prolactin. The addition of prolactin shifted the hLH dose–response curve to the right and depressed the maximal response in comparison to the curve without prolactin. Finally, inhibitory doses of prolactin resulted in no detectable change in LH receptor number as estimated from Scatchard plots. It is concluded that prolactin inhibits LH action on interstitial cells as determined by rate of testosterone production except at very large doses of prolactin where LH action is less inhibited or augmented. The inhibitory action of prolactin in this *in vitro* interstitial cell assay was not accompanied by a decrease in LH receptor number. Thus, a postreceptor action is likely to be involved.

Hyperprolactinemia is now recognized as a common cause of infertility in women, and an occasional cause of decreased libido and infertility in men. The mechanisms by which prolactin interferes with reproduction are uncertain, and considerable, seemingly conflicting data exist in published studies. In two previous studies, Patritti Laborde and Odell (1) and Larsen and Odell (2) evaluated the effects of acute elevations of prolactin in male rabbits on several facets of the endocrine reproductive system. These studies showed that (a) prolactin did not modify the LH/FSH response to a single injection of GnRH; (b) prolactin suppressed or inhibited the testosterone response to both exogenous hCG and endogenous LH; (c) prolactin prevented the expected rise in endogenous LH and FSH in response both to lowered testosterone in intact animals and to castration; and (d) prolactin decreased the pulse amplitude of LH and FSH, and decreased mean LH and FSH, but did not change pulse duration in intact male rabbits. In the present studies, we have attempted to better characterize the prolactin inhibition of LH stimulation of testosterone, by studying the phenomenon *in vitro*, using Leydig cells from both rats and mice.

Methods. The *in vitro* Leydig cell (interstitial cell) bioassay described by Moyle and Ramachandran (3) was used with cell preparations from both rats and mice. Inter- and intraassay coefficients were 12 and 4.4%, respectively, for the rat assay; for the mouse assay they were 11.9 and 3.9%. The prolactin preparation employed in all studies was NIH bovine prolactin (B-6), which was kindly supplied by the NIAMDD hormone distribution program. This preparation of prolactin contains less than 0.5% LH or FSH activity by weight. The human LH preparation employed in all studies was immunochemical grade hLH (AFP 6332B-NIAMDD-hLH-I-2), also kindly supplied by NIAMDD. Testosterone was measured by a radioimmunoassay (RIA) employing an antiserum, kindly supplied by Dr. Wayne Meikle, which

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was produced in rabbits by immunization with a BSA-3-testosterone conjugate (antiserum M-84). The separation reagent was charcoal (0.2% charcoal, 0.02% dextran). Interassay and intrassay coefficients of variation were 6 and 10%, respectively, for the testosterone RIA. For the studies where LH receptor numbers were calculated, hLH was radioiodinated using Concanavalin A purification, as previously described by Patritti Laborde *et al.* (4). Receptor numbers were estimated using Scatchard plots. For these estimates four independent experiments were performed; complete hLH dose-response curves were constructed in the presence of no prolactin and with 33 ng/ml of prolactin.

The interstitial cells were resuspended in Krebs-Ringers buffer containing 0.2% BSA and 0.1% lima bean trypsin inhibitor (Sigma Chemical Co.), for a total volume of 3 ml in each 24-ml Erlenmeyer flask. The flasks receiving prolactin were preincubated 30 min before all flasks received varying doses of hLH. Both prolactin and control flasks were then incubated for an additional 90 min, at 37°C, in an atmosphere of 95% oxygen and 5% carbon dioxide, with continuous shaking in a Dubnoff shaker.

Results. Figures 1A and B show the mean dose-response curves for hLH in the mouse and rat Leydig cell bioassays, respectively. From these data, a small, near threshold dose of hLH was selected for the bioassay using each species of Leydig cell. For the mouse, 167 pg/ml and, for the rat, 500 pg/ml were selected. In the presence of this constant hLH dose, the dose response to prolactin was evaluated. Figures 2 and 3 show the biphasic dose-response curve in two independent experiments using mouse Leydig cells (Fig. 2) and rat Leydig cells (Fig. 3). Doses of prolactin between 0.33 and 33 ng/ml produced a progressive inhibition of LH-stimulated testosterone. Larger doses of prolactin produced less inhibition, or even an augmentation of LH action. In these and four additional studies (not shown) the effect of large doses of prolactin was variable, returning testosterone to baseline or above baseline.

Complete dose-response curves for hLH were then constructed in the presence of three doses of prolactin. Figure 4 shows these data. Note that increasing doses of prolactin shifted hLH dose-response curves to the right (when plotted on semilog graphs), and that larger doses lowered the maximal response to hLH. Table I summarizes four independent experiments showing the effect of 1 μg/ml prolactin on hLH receptor number. In each of the four experiments, testosterone secretion into medium was shown to be inhibited by the added prolactin. Prolactin

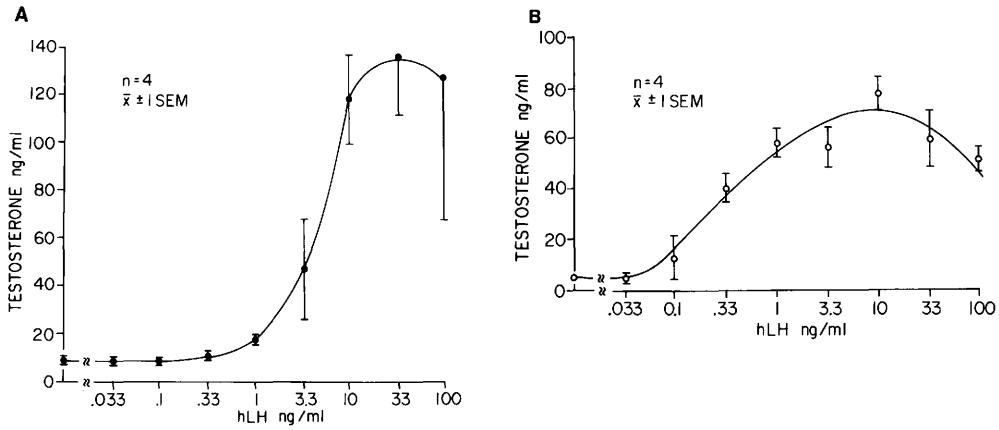

FIG. 1. Mean dose-response curves from human LH (hLH) stimulation of testosterone production by interstitial cells *in vitro*. (A) Mean curve of four independent assays using rat interstitial cells. The brackets enclose 1 SEM. (B) Mean curve of four independent assays using mouse interstitial cells. Average inter- and intraassay coefficients of variation for the mouse assay was 11.9 and 3.9%, respectively. For the rat assay they were 12 and 4.4%, respectively.

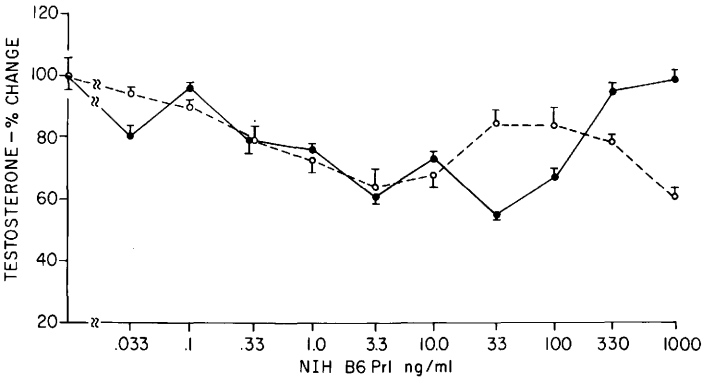

FIG. 2. Effects of varying doses of prolactin on the stimulation of testosterone secretion by mouse interstitial cells produced by a constant dose of 167 pg/ml hLH. Two independent experiments are shown (solid and dashed lines). Brackets enclose 1 SEM.

did not produce any detectable changes in LH receptor number, although it did inhibit its stimulation of testosterone secretion.

The effects of prolactin alone (in the absence of LH) are shown in Fig. 5. These data show that no detectable LH activity is present in this prolactin preparation in doses up to 1000 ng/ml. Furthermore, in the absence of LH, prolactin did not decrease testosterone secretion.

Discussion. Some published studies have indicated that prolactin may interfere with gonadotropin action in both humans and rats. In men, hyperprolactinemia is associated with decreased blood testosterone and "normal" or low blood LH and FSH. In women, hyperprolactinemia produces amenorrhea and hypoestrogenism, with low or normal LH and FSH. Mroueh and Siler-Khodr (5) showed that women with hyperprolactinemia were resistant to ovulation induction with the usual doses of human menopausal gonadotropin and human chorionic gonadotropin. These same workers showed that such women also failed to respond to Clomid, an antiestrogen that produces a rise in LH and FSH in normal men and women. Demura *et al.* (6) recovered ovarian tissue from

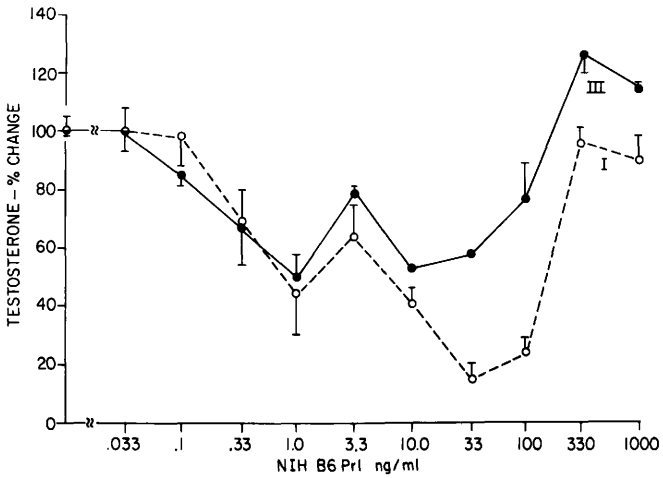

FIG. 3. Effects of varying doses of prolactin on the stimulation of testosterone by rat interstitial cells produced by a constant dose of 500 pg/ml of hLH. Two independent experiments are shown (solid and dashed lines). Brackets enclose 1 SEM.

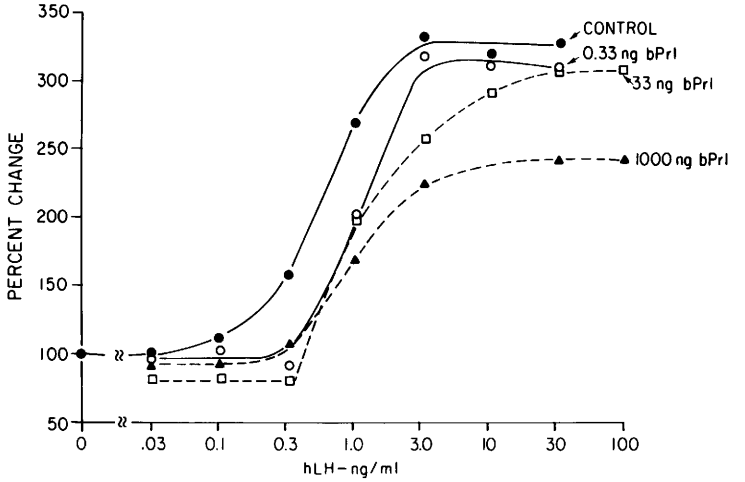

FIG. 4. Dose–response curves for hLH in the presence of varying doses of prolactin by rat interstitial cells. Note that the hLH dose–response curves are shifted to the right and the maximum response is depressed by prolactin. Each point represents the average at triplicate determinations.

women undergoing oophorectomy and studied hCG stimulation of estradiol and progesterone *in vitro*. In the presence of ovine prolactin, added to the incubation media, the effects of hCG were inhibited. Arafah *et al.* (7) studied two men with hyperprolactinemia and demonstrated that hCG had little effect in stimulating testosterone secretion.

Studies in rats are numerous. In 1977, Grandison *et al.* (8) reported that the postcastration rise in LH and FSH in both male and female rats was prevented by an injection of exogenous prolactin or by implantation of prolactin into the median eminence of the hypothalamus. In 1978, Winters and Loriaux (9) reported that hyperprolactinemia (produced by ectopic pituitary glands) prevented the LH and FSH rise at 1 and 3 days following castration. Login *et al.* (10) in 1982 confirmed that the postoophorectomy rise in LH was prevented by hyperprolactinemia in rats, which was produced by transplantable, prolactin-secreting tumors. Fang *et al.* (11) showed that in male rats, after several months of hyperprolactinemia caused by a transplantable, prolactin-producing tumor, a low testosterone and severe testicular atrophy resulted.

TABLE I. EFFECTS OF PROLACTIN ON LH RECEPTOR NUMBER IN RAT INTERSTITIAL CELLS

Expt	Percentage change prolactin versus control
1	−1
2	+13
3	−18
4	+13
$\bar{X}$	+1.8%

Note. Dose–response curves for hLH were performed in the presence of 33 ng/ml prolactin. The hLH receptor number per cell was estimated from Scatchard plot analysis and Leydig cell number. Mean hLH receptor number in different experiments round from 100,500 to 183,600/cell.

In contrast, Advis and Ojeda (12) produced hyperprolactinemia in female rats by treatment with sulpiride (a dopaminergic receptor blocker) and noted precocious vaginal opening. They concluded that prolactin sensitized the ovaries to low prepubertal levels of gonadotropins. These workers demonstrated *in vitro* that estradiol and progesterone secretion in response to hCG *in vitro* were enhanced when ovaries were obtained from hyperprolactinemic animals. However, in these studies, prolactin was not added *in vitro*. The duration of endogenous prolactin effects when tissues are removed and incubated *in vitro* is not known.

Bartke and Dalterio (13, 14) and Bex and Bartke (15) also suggested that prolactin may augment LH-stimulated testosterone secretion

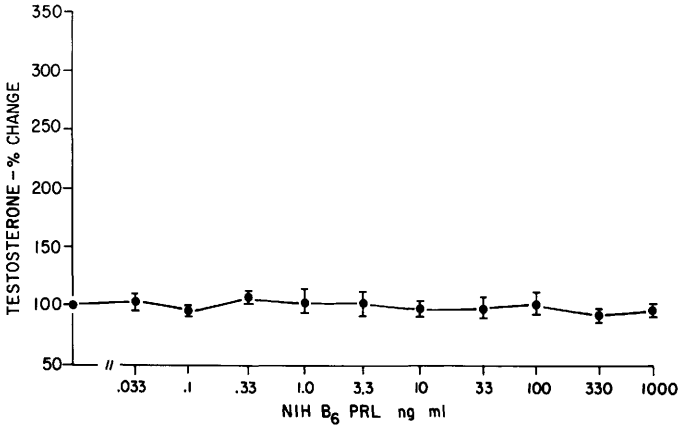

FIG. 5. The effects of varying doses of prolactin on basal testosterone secretion by rat interstitial cells in the absence of LH. These data show that prolactin in doses up to 1000 ng/ml contains no detectable LH activity and that prolactin does not change testosterone secretion in the absence of LH. $N = 4$; $\bar{X} \pm 1$ SEM.

in rats and hamsters. Bex and Bartke (15) showed that prolactin increased LH receptor number. In contrast to these studies in hypophysectomized animals, Odell and Swerdloff (16), using a wide range of doses of exogenous prolactin in hypophysectomized male rats, reported that prolactin did not augment LH action on the testes. However, they reported that prolactin given with growth hormone augmented a growth hormone-stimulated increase in LH action on the testes. In 1961, Woods and Simpson (17) reported that prolactin stimulated spermatogenesis in hypophysectomized male rats. Furthermore, in prolactin/growth hormone-deficient dwarfed mice, testicular and Leydig cell function are greatly impaired, causing sterility. Treatment of these animals with prolactin restores fertility; treatment with GH also restores fertility (14).

In recent studies using the male rabbit *in vivo,* we have attempted to more systematically study the effects of hyperprolactinemia (1, 2). These studies showed:

1. Acute hyperprolactinemia produced by bovine prolactin did not alter the LH/FSH response to a single injection of GnRH.

2. The testicular testosterone response to increased endogenous LH or to exogenous hCG was impaired by acute hyperprolactinemia.

3. We confirmed that the postcastration rise in LH/FSH was prevented by prolactin.

4. Prolactin decreased the amplitude of both LH and FSH pulses, and decreased mean LH and FSH, but did not change pulse duration.

In the current studies, we evaluated in greater detail the effects of prolactin on Leydig cell function. We employed rat and mice Leydig cells because of easier availability and our past experience in using these Leydig cells in bioassay. These studies have shown that, depending on dose, prolactin can both inhibit and augment LH stimulation of testosterone. These studies also show that prolactin shifted the dose–response curves for prolactin rightward and decreased the maximal LH response. At doses of prolactin that inhibited LH action stimulated testosterone secretion, no change in LH receptor number could be detected.

The biphasic dose–response curve of prolactin modulation of LH action may assist in explaining some previous conflicting reports on the prolactin effects on testicular function. The variability in our data do not permit determination of whether prolactin at very high doses returned testosterone secretion to control levels or stimulated secretion to above control levels.

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