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Effects of Prolactin on Luteinizing Hormone-Stimulated Testosterone Secretion in Isolated Perfused Rat Testis

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The direct peripheral effect of prolactin on LH-stimulated testosterone secretion was re-evaluated using the intact, isolated, perfused rat testis. In paired experiments, one testis was infused with the hormones being studied; the other testis of the same rat was used as the control. A dose-response curve of LH-stimulated testosterone secretion was established first. A dose of 300 ng of LH, which was on the ascending portion of the dose-response curve, was selected so that both stimulatory and inhibitory effects of prolactin could be observed. Prolactin doses ranging from 0 ng to 3000 ng were then tested to determine alterations in LH-stimulated testosterone secretion. Prolactin inhibited LH-stimulated testosterone secretion at low doses (< 300 ng), but augmented LH action at high doses (> 1000 ng). These results showed that prolactin and LH interact with each other in a biphasic dose-dependent manner.

Key words; prolactin, testes, luteinizing hormone, testosterone secretion.

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Hyperprolactinemia is a major cause of infertility in humans of both sexes. In men, hyperprolactinemia is associated with decreased libido, decreased blood testosterone, and "normal" or low blood LH and FSH concentrations (for review, see Evans et al, 1982). However, when the effects of hyperprolactinemia are studied in rodent animal models, particularly rats and mice, contradictory results have been obtained. Some reports have indicated that prolactin (PRL) caused an augmentation of testosterone (T) secretion (Woods and Simpson, 1961; Bartke, 1971a; 1971b; Bartke and Dalterio, 1976; Bartke et al, 1977a), while others have shown that PRL had an inhibitory effect (Fang et al, 1974; Grandison et al, 1977; Winter and Loriaux, 1978). In a previous study from our laboratory employing isolated Leydig cells from rats and mice incubated *in vitro* (Odell and Larsen, 1984), we reported that prolactin caused a biphasic effect on T secretion. Prolactin inhibited LH-stimulated secretion of T at doses of ≤100 ng/ml, while at higher doses it stimulated T secretion. Because we were concerned that *in vitro* studies of Leydig cells might not mimic actual physiologic conditions and that the isolation process itself may cause some cell damage, we attempted to re-evaluate the

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effect of PRL on LH-stimulated T secretion using the intact, isolated, perfused rat testis.

Materials and Methods

Materials and Equipment

Bovine PRL (USDA-bPRL-B1) (< 0.5% by wt. LH contamination as reported from USDA data) and ovine LH (NIH-oLH-S17, PRL contamination < 0.1% by wt based on NIADDK data) were supplied by the National Hormone and Pituitary Program (Bethesda, MD). We have independently evaluated possible LH contamination of the bovine PRL preparation using the *in vitro* rat Leydig cell assay. Prolactin produced no increase in basal T secretion in doses up to 3000 ng, the highest dose used in our studies. Bovine serum albumin (BSA; Sigma A4503) (<12 pg/mg PRL contamination and undetectable LH activity by our direct assay) (Lot #37F- 0816), activated charcoal, and dextran were supplied by Sigma Chemical Company (St. Louis, MO). [1,2,6,7-^{3}H (N)T was obtained from New England Nuclear Company (Albany, NY) (NET-370), while the T antibody was supplied by Endocrine Science Company (Tarzana, CA). Krebs Ringer bicarbonate buffer (0.154 M NaCl, 0.154 M KCL, 0.11 M $CaCl_2$, 0.154 M KH_2PO_4, 0.154 M $MgSO_4$.7OH, and 0.154 M $NaHCO_3$) was prepared fresh for each set of experiments and used within 2 days, making unnecessary the addition of an antibiotic.

Perfusion Methods

The perfusion equipment and methods (Fig. 1) followed the basic design of Vandemark and Ewing (1963), and Chubb and Desjardins (1983a) with minor modifications. Krebs Ringer bicarbonate buffer with 3% w/v BSA was gassed with 95% O_2 and 5% CO_2 starting 1 hour before and continuing throughout the entire experiment. Both the buffer and the funnels containing the isolated organs were maintained at a constant temperature of 32 ± 1 C in a water bath.

A mature male Sprague Dawley rat (200 to 250 g) was anesthetized by anhydrous ethyl ether and each testis was removed. A small catheter was inserted into the capsular artery of each testis and secured by suture and glue. The catheter was flushed with less than 100 µl of 100 U/mL heparin. The catheters were then connected to the tubings of the peristalic pump that infused the Krebs Ringer's bicarbonate buffer with 3% w/v BSA at a rate of 10 ml/hour.

Fifteen-minute samples were collected continuously for 3 hours. The first two samples from each testis served as baseline for T concentrations to ensure no difference in baseline T production between the paired organs. After 0.5 hour, a selected dose of ovine LH in a volume of 0.1 ml of normal saline was infused randomly as a bolus through the 3-way stop-cock into the catheter of one testis, while an equal volume of normal saline was infused into the contralateral testis as control. These studies produced the LH-T dose-response relations.

After establishing the dose-response curve of oLH versus T production, a dose of 300 ng of LH that was on the ascending portion of the dose-response curve was selected.

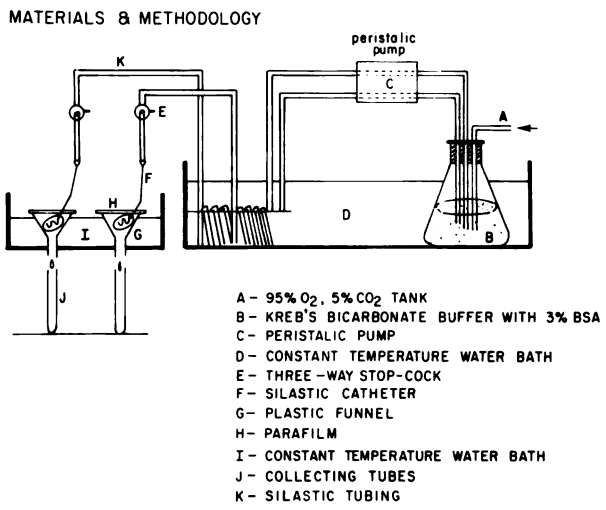

Fig. 1. Set up of the experiment. The perfusion equipment followed the basic design of Vandemark and Ewing (1963) and Chubb and Desjardins (1983a), with slight modification. Krebs Ringer bicarbonate buffer with 3% w/v BSA was gassed with 95% O_2 and 5% CO_2 starting 1 hour before and continuing throughout the entire experiment. Both the buffer and each funnel containing the isolated testis were maintained at a constant temperature of 32 ± 1 C in a water bath. A peristalic pump was used to perfuse each testis at 10 ml/hour/testis. oLH and bPRL were injected as a bolus through the three-way stop-cock into the perfusate as described in the Materials and Methods section.

The effects of varying doses of bovine PRL on LH-stimulated secretion of T were than determined. The procedures were similar to those mentioned above, except that different concentrations of bPRL in a volume of 0.1 ml normal saline were infused as a bolus into one testis at the beginning of the experiment while the contralateral testis received 0.1 ml normal saline as the control. After 0.5 hour, 300 ng of oLH in 0.1 ml normal saline was injected as a bolus into both organs. Fifteen-minute samples were collected as described above. (A total of 3 hours of 15-minute samples were collected, including the first 0.5 hour as control.)

Testosterone (Radioimmunoassay)

Radioimmunoassay was performed as follows: 100 µl of each 15-minute sample were pipetted in duplicate into test tubes containing 700 µl phosphate buffered saline (0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4), 100 µl 10^5 cpm per ml of [^{3}H]T, and 100 µl 1:1000 T antibody. The tubes were vortexed and continuously shaken in a shaker at 4 C for at least 3 hours. Two hundred µl of 1% w/v activated charcoal/0.1% w/v dextran in phosphate buffered saline were then added to each tube; the tubes were vortexed, allowed to stand at 4 C for 15 minutes, and then centrifuged at 2000 rpm for 20 minutes. A 0.5-ml aliquot was taken from each sample and mixed with 5 ml of scintillation fluid and analyzed in a model LS-330 Beckman liquid scintillation counter.

The radioactivity (cpm) of each sample was compared to

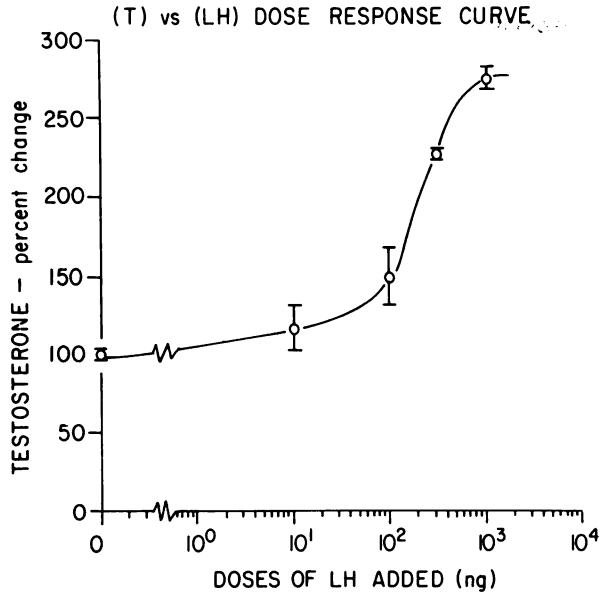

Fig. 2. Mean dose-response curve from ovine LH stimulation of T production by the isolated perfused rat testis. The results are expressed as the average of two animals (± 1 SEM). The results show a dose-response relationship of increasing T production when the concentration of oLH is increased.

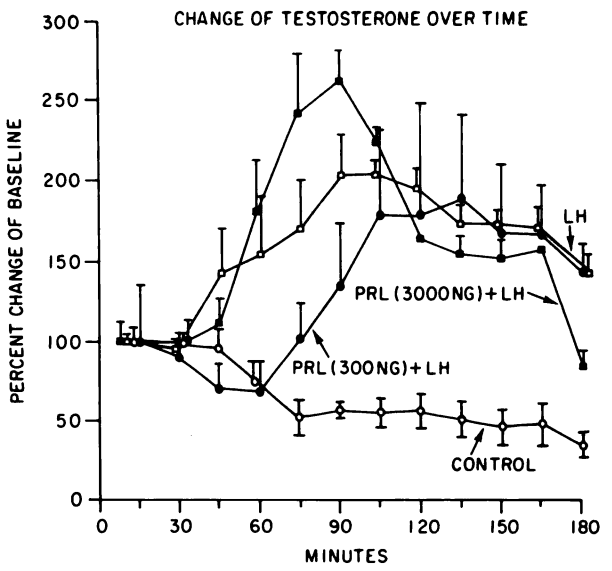

Fig. 3. The time course of the change in T in response to 300 ng of LH (open boxes) compared to zero LH (control; open circles), and as modified by 300 ng (closed boxes) and 3000 ng (closed circles) of PRL.

the standard curve and the corresponding concentration of T in each sample was calculated by a computer program. Results were plotted by a cricket-graph program developed by Cricket Software, Inc. (Philadelphia, PA).

Calculation of Percent Change

The % change value is based on the total amount of T produced by the experimental testis in the entire 3 hrs (as expressed by the area under the curve), divided by the total amount of T produced by the control, the latter being 100%.

For the initial studies of LH-T dose-response relations, we compared LH alone (experimental) versus baseline T production (control). The area under the curve of the former was divided by that of the latter and multiplied by 100. Similarly, in the second series of experiments, we compared LH-stimulated T production in the presence of PRL (experimental) versus LH without PRL (control). The percent change is calculated as above using the area under the curve.

Left and Right Comparison and Intraassay and Interassay Accuracy

Before we began our experiments, we were concerned that there might be an intrinsic difference between the left and right testis in baseline T secretion. Therefore, we compared the baseline T secretion of each paired testis during the first 0.5 hour of each experiment to ensure that there was no difference between the two sides. In addition, we assessed the difference in T production between the left and right testis stimulated by 300 ng of LH in four rats. Statistical analysis by paired *t*-test showed there was no significant difference ($P > 0.4$) in the LH-stimulated T secretion between the left and the right testis.

Quality control of RIA results was ensured by determining the percent coefficient of variation (% CV) within assay and between assay. The average % CV of the interassay accuracy was 9.2% ± 1.4. The intraassay accuracy of five assays chosen randomly showed an average % CV of 4.6% ± 1.6.

Results

Figure 2 shows the effect of varying doses of LH on T secretion. Analysis of variance by the F-test showed the differences were statistically significant ($P < 0.0005$). Increasing doses of LH (up to 1000 ng) increased T secretion. To study the effect of PRL on LH-augmented secretion of T, we chose a dose on the ascending portion of this dose-response curve, 300 ng of LH. By doing this, we could observe both the stimulatory and inhibitory effects of PRL.

Figure 3 shows the temporal relationship of LH alone and LH together with high (3000 ng) and low doses (300 ng) of PRL to T secretion. The saline control is also included. It is clear that LH in the presence of a high dose of PRL augments secretion of T. In contrast, an inhibitory effect is seen with the low dose. Other doses have similar temporal relationships and are not included in the graph for the sake of clarity.

Figure 4 shows the effects of varying doses of PRL

on LH-stimulated T secretion. At PRL doses ranging from 1 to 300 ng, LH-stimulated T secretion was inhibited, while doses of 1000 and 3000 ng augmented T secretion.

The 0-ng dose of PRL showed a mean of 101 ± 4.8% and was used as baseline to compare with the means of other doses. Global analysis of variance of all doses by F-test had a significance level of $P < 0.005$. When individual doses are compared with the baseline by unpaired *t*-test, 1-ng ($P < 0.005$), 100-ng ($P < 0.01$), and 1000-ng doses ($P < 0.025$) were statistically significant. The remaining doses were either at the turning point of the curve or have a greater standard error of the mean and therefore were not statistically different by this analysis.

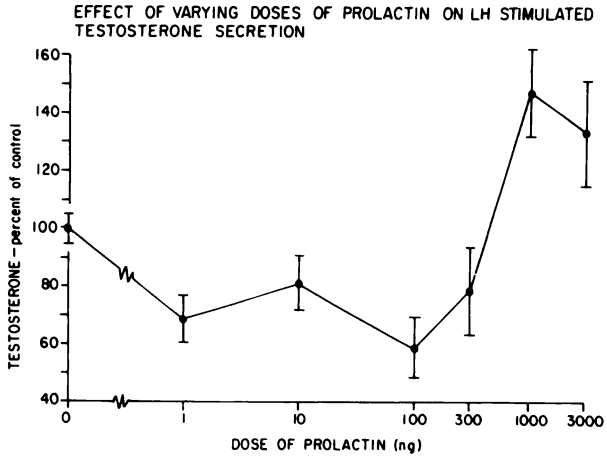

Fig. 4. Effects of varying doses of bovine PRL on the stimulation of T by the isolated, perfused rat testis produced by a constant dose of 300 ng of oLH. Experimental results from four animals were pooled per dose (± 1 SEM). Note the inhibition of oLH-stimulated T secretion by bPRL at dose less than 300 ng. A stimulatory effect is seen when doses are at 1000 to 3000 ng of bPRL.

Discussion

In humans, hyperprolactinemia-induced infertility in both sexes is well documented (Evans et al, 1982). However, animal studies of the action of PRL on reproduction have had contradictory results. In 1961, Woods and Simpson reported that PRL stimulated spermatogenesis in hypophysectomized male rats. In addition, they showed that in PRL/GH-deficient dwarf mice, testicular and Leydig cell functions were impaired and treatment with either PRL or GH could restore fertility. Bartke (1971a, 1971b), Bartke and Dalterio (1976), Bartke et al (1977a), and Bex and Bartke (1977) reported that PRL augmented LH-stimulated secretion of T in rats and hamsters. Prolactin was also shown to act synergistically with T on male sex accessory glands in castrated rats and mice (Bartke and Lloyd, 1970; Moger and Geschwind, 1972; Kennan and Thomas, 1975; Bohnet and Friesen, 1976; Bartke et al, 1977b). In addition, Suescun et al (1985) showed that hypoprolactinemia induced by bromocriptine during gonadal maturation of male rats caused a decrease in both basal and hCG-stimulated T secretion.

On the other hand, Hartmann et al (1966) reported there was a decrease in copulatory behavior in male rabbits after PRL administration. Fang et al (1974) showed that PRL-secreting tumors produced low blood levels of T and severe testicular atrophy in male rats. Moreover, several other studies such as Grandison et al (1977) and Winters and Loriaux (1978) reported that hyperprolactinemia prevents the postcastration rise in LH/FSH in male rats, which indicated that PRL may act via preventing a feedback increase in LH/FSH during low blood levels of T. Other studies from our laboratory (Laborde and Odell, 1984) demonstrated in male rabbits that single intravenous injections of PRL lowered blood T. Furthermore, PRL was found to inhibit hCG-stimulated T secretion by a direct gonadal inhibitory action. Prolactin did not alter GnRH, or stimulation of LH secretion, but did prevent the postcastration rise in LH and FSH in rabbits.

In detailed studies of LH pulsatile characteristics in male rabbits, Larsen and Odell (1984) showed that PRL infusion slowed LH pulse frequency and lowered mean LH concentrations. In a separate study, Odell and Larsen (1984) demonstrated a biphasic effect of PRL on LH-stimulated T secretion in purified Leydig cells of both mice and rats.

Prolactin receptors were first identified in rat Leydig cells in 1977 by Charreau et al. Although Bex and Bartke (1977) later showed that PRL increased LH receptors in rats, Odell and Swerdloff (1976) did not find an increase in LH receptors in PRL-treated hypophysectomized male rats. On the contrary, Ota et al (1986) reported that a single injection of 0.2 to 5 IU of PRL would decrease testicular LH receptors and would increase PRL receptors.

In addition, timing is very important in observing a difference in the effect of PRL. Chan et al (1981) reported that LH increased PRL receptors initially (first 1 to 2 hours), but would deplete them later (4 to 6 hours). Barkey et al (1987) showed that PRL (50 to 1000 ng/ml) inhibited basal or LH-stimulated T secretion by Leydig cells in a 3-day culture but had no

effect in the short-term, 4-hour culture. Ota et al (1986) reported that PRL induced a decrease in serum T during day 1; a return to normal during day 2; and an increase on day 3. These results showed that PRL and LH can affect each others' receptors in a sensitive dose-related/time-related manner that is poorly understood.

Prolactin can affect T secretion either centrally by effects on the hypothalamus/pituitary axis or peripherally by acting directly on the testis. In this study, we have attempted to use a different technique to study the direct gonadal affects of PRL on LH-stimulated T secretion. Perfusing the isolated testis with Krebs Ringer buffer is a well established technique to study testicular function (Ewing and Eik-Nes, 1966; Ewing et al, 1975; 1979; Baker et al, 1977; Chubb and Ewing, 1979; Lee and Nagayama, 1980; Cobb et al, 1980; Darney and Ewing, 1981; Hoffer et al, 1983; Chubb and Desjardins, 1983a; 1983b; and Thompson et al, 1983). Another advantage of perfusing an isolated testis is the elimination of the effect of endogenous PRL in the blood that can affect the results.

After we established the dose-response curve of the LH effect on T we chose a 300-ng dose that is on the ascending limb of the dose-response curve. We then studied the effects of different doses of PRL on LH-stimulated T secretion. The results show a similar biphasic inhibitory/stimulatory effect for isolated Leydig cells as reported previously by Odell and Larsen (1984). Unfortunately, it was shown in the earlier studies that the viability of the isolated perfused testis will decrease after 2 to 3 hours of perfusion (Baker et al, 1977; Cobb et al, 1980; Chubb and Desjardins, 1983a), making unreliable the perfusion of the testis for the longer period of time needed for detecting further changes suggested by some of the studies mentioned above.

To summarize, PRL causes an inhibitory effect of LH-stimulated T secretion at low doses and a stimulatory effect at high doses. Whether these effects are mediated by LH/PRL receptor interaction or via interference with T synthesis is not known. However, these *in vivo* studies add further evidence that PRL modulates LH-stimulated T secretion in a biphasic manner. These studies emphasize that when PRL effects are reported, an extensive dose-response curve is necessary.

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