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Original Article

HIGH DOSES OF PROLACTIN INHIBIT TESTOSTERONE SECRETION IN RAT LEYDIG CELLS

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[1] **The effect of prolactin on dispersed rat Leydig cells was investigated. Leydig cells from adult rat testes of proven fertility were isolated via collagenase digestion and dispersion. About 100,000 Leydig cells/ml were incubated with graded concentrations of prolactin in the presence or absence of luteinizing hormone (LH) and theophylline for 3hr. at 37°C. Leydig cell viability was assessed by trypan blue dye exclusion method. The reaction was terminated by the addition of cold (4°C) Dulbeccos's modified Eagle's medium (DME) and testosterone in suitable aliquots was estimated by a validated radioimmunoassay technique.**

Prolactin inhibited *in vitro* Leydig cell steroidogenesis at doses of 1.25-10.00 i.u/L but only potentiatated the effect of LH at doses ranging between 0.31 and 0.62 i.u/L. LH had no effect on prolactin (2.5 - 10.0 I.U/L) inhibited testosterone secretion. Theophylline (10mM/L) did not produce any stimulatory effect on prolactin - inhibited testosterone seretion by Leydig cells *in vitro*. At lower doses of prolactin, theophylline was significantly lower (P<0.01) than that produced by LH at lower doses of prolactin. The results showed that high doses of prolactin inhibited testosterone secretion in rat Leydig cells and the mechanism of action of prolactin on Leydig cell steroidogenesis could be via cAMP second messenger system.

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INTRODUCTION

Prolactin is a peptide hormone produced in the anterior pituitary gland. Since being differentiated as a separate hormone from growth hormone in primates (Frantz and Kleinberg, 1970), prolactin has been recognised as an important regulator of fertility as well as lactation in the human (Yen and Jaffe, 1986). In keeping with the numerous actions of prolactin on metabolic and reproductive processes, specific receptors for the hormone have been found in the liver, the lung, and in the adrenal, prostate and mammary gland as well as in the testis and ovary (Posner *et al.*, 1974). The hepatic prolactin receptors are marked by steroid- dependent, being increased by estrogen as well as Prolactin itself, and reduced by androgens (Ranke *et al*, 1976)

The early suggestions that prolactin can act directly on the male reproductive system received strong support from the demonstration that specific Prolactin receptors are present in the male accessory reproductive glands (Kledizik *et al*, 1976) and in the interstitial compartment of the testis (Aragona *et al*, 1977; Charreau, *et al*, 1977). It has been documented that prolactin can potentiate

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the effects of exogenous androgens on the growth of male accessory reproductive glands in castrated animals (Thomas and Keenan, 1976). The findings of Sheth *et al* (1975) that prolactin is present in human ejaculates and that prolactin levels in seminal plasma are higher than those in the serum with seminal vesicles contributing the highest concentration were confrimed in several laboratories (Dericks-Tan *et al.* 1978; Krause, 1978; Segal *et al*, 1978).

However, the mechanism of prolactin action in the reproductive glands has not been established. Numerous studies indicate that prolactin can increase cytoplasmic and nuclear uptake of testosterone and dihydrostestosterone in prostatic tissue (Fansworth, 1972; Barker *et al*; 1977; Johansson, 1978), but no studies of prolactin effects on androgen receptor levels in accessory reproductive glands have been reported.

The mechanism responsible for the stimulation of testicular function by Prolactin was suggested by the results obtained in hypophysectomized rats and mice where prolactin significantly augmented the effects of luteinizing hormone (LH) on biosynthesis of tyestosterone and spermatogenesis (Bartke, 1971). Bartke *et al*, (1978) demonstrated that treatment of hypophysectomized rats with prolactin increases their ability to produce testosterone in response to acute LH stimulation. These results suggest that prolactin can act on the Leydig cells to increase their responsiveness to LH stimualation. This suggestion is however based mostly on *in vivo* reports in both experimental animals and man (Bartke, 1980). The available in vitro reports are conflicting. While Odell *et al* (1974) reorted that prolactin had no effect on basal testosterone secretion by Leydig cells in short – term culture. It should be noted that these studies (Odell *et al*, 1974; Barkey *et al*, 1987) used doses of prolactin ranging between 50 and 1000ng/ml. In view of the association of hyperprolactinemia in men with loss of libido and impoternce (Thorner and Besser, 1978), further studies on the role of prolactin in male reproduction become worthwhile. The present paper describes the action of various doses of prolactin on Leydig cell steroidogenesis *in vitro*.

MATERIALS AND METHODS

Animals

Wistar strain albino rats (250-300g) obtained from the central animal house, College of medicine, University of Ibadan were used for the study. The rats were housed individualy in wire mesh cages under controlled light (12L: 12D cydes) and temperature (24 ± 1°C) conditions. Each rats was certified fertile by isolated mating technique (Raji and Bolarinwa, 1997).

Hormones, Drugs and Reagents

Human luteinizing (LH), Prolactin, testosterone antiserum, testosterone standard and ^{3}H-testosterone were obtained from World Health Organisation Matched Reagent Programme and made up in phosphate buffered saline (PBS, PH 7.4) when needed. Collagenase, type I (Clostridium hystolyticum), Dulbecco's modified Eagle's medium (DME) containing glucose, L-glutamine and without phenol red and sodium bicarbonate, bovine serum albumin (BSA); lima bean trypsin inhibitor; trypan blue dye; activated charcoal, dextran; PPO 2,5-diphenyloxazole and POPOP, 1, 4-bis(2- (5-phenylozolyl) benzene, phenyl – oxyzolyl phenyl, were obtained from Sigma chemical company, St. Louis Missouri USA. All organic solvents and mineral acids were of the analytical grade. Theophylline (Powder) was obatined in pure form from Mermaid Pharmacy, inc, Brooklyn, New York.

Experimental Procedure

Experiments were performed with dispersed Leydig cells isolated from normal untreated rats testes as previously described (Raji 1995; Raji and Bolarinwa 1997). Briefly, Leydig cells from adult rats (250-300g) were prepared by collagenase digsestion followed by gentle dispersion. Groups of two decapsulated testes were minced and carefully kept in 20mls plastic tubes. The digestion with collagenase (1mg/ml, 37°C, 20mins) was carried out in Dulbesso's modified Eagle's medium (DME) containing 0.1% BSA amd

1mg/10ml trypsin inhibitor. The cell suspension was washed with 5ml DME by centrifugation at 200*g* 4°C for 5 minutes. The cell pellet was immediately suspended in 5ml DME and aspirated at 20,000g, 4°C for 60 minutes and the supernatant decanted. The cell pellet was again resuspended in 5ml DME and aspirated with Pasteur pipette.

Leydig cell viability was assessed by trypan blue dye exclusion test. Only fractions with more than 90% viability were employed for the study. 0.1ml of the cell suspension was incubated separately with 0.1ml phosphate buffered saline (PBS), LH, and Prolactin alone, Prolactin plus theophylline and Prolactin plus LH. Prolactin was administered in doses ranging from 0.31 – 10.0 i.u/L. Hormone and drug solutions were prepared in PBS and reaction was stopped by addition of 4ml cold DME each time. Testosterone in suitable aliquots of unextracted samples was estimated after appropriate dilutions by a validated radioimmunoassay (RIA) technique.

Statistical Analysis

This was done by the Student's t-test and ANOVA

RESULTS

In this study, the inter- and intra-assay variations for testosterone were 9.5 ± 0.95% and 10.20 ± 1.50% respectively.

Fig. 1 shows the effect of graded doses of LH in the presence or absence of 10mn theophylline on testosterone secretion by Leydig cells in vitro. There was an initial dose- dependent increase in testosterone secretion up to 25ng/ml LH ut a remained constant at higher doses of LH. Addition of theophyline produced significant ($P<0.05$) increases in LH-induced testosterone secretion.

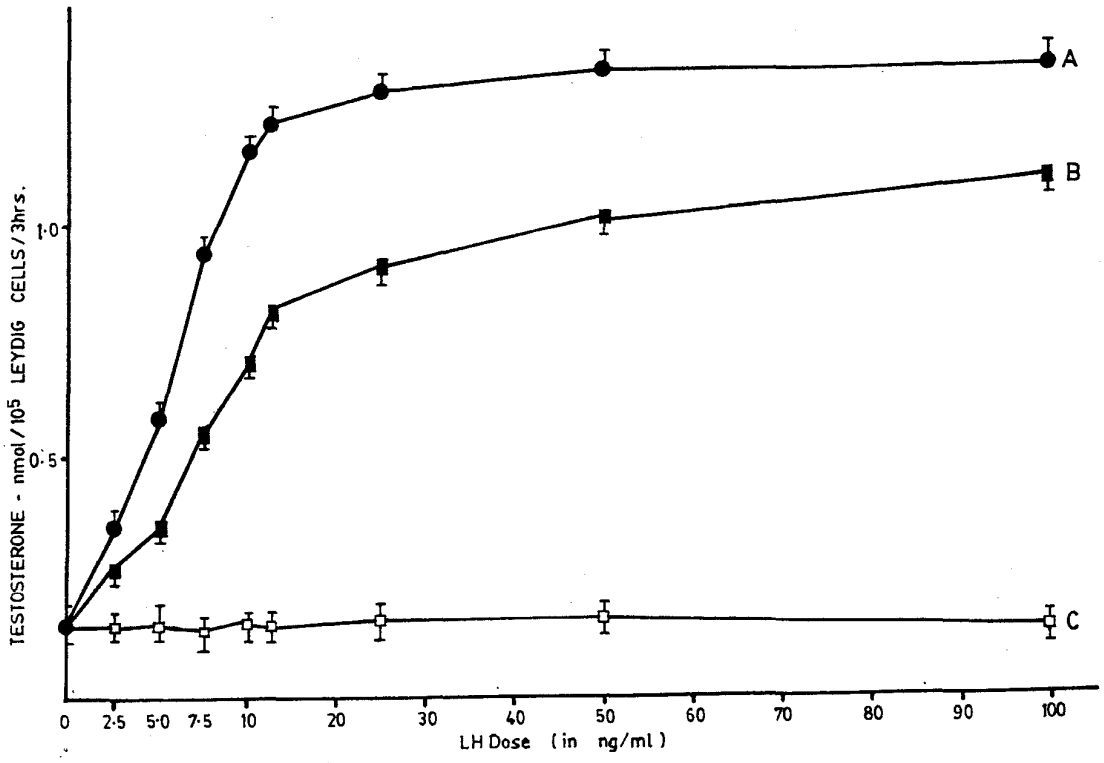

Fig. 1.
Effect of Luteinizing hormone (LH) on Testosterone secretion in Leydig cells *in vitro*. A =LH + 10mM Theophyline; B =LH alone; C= Phosphate buffered Saline (PBS).

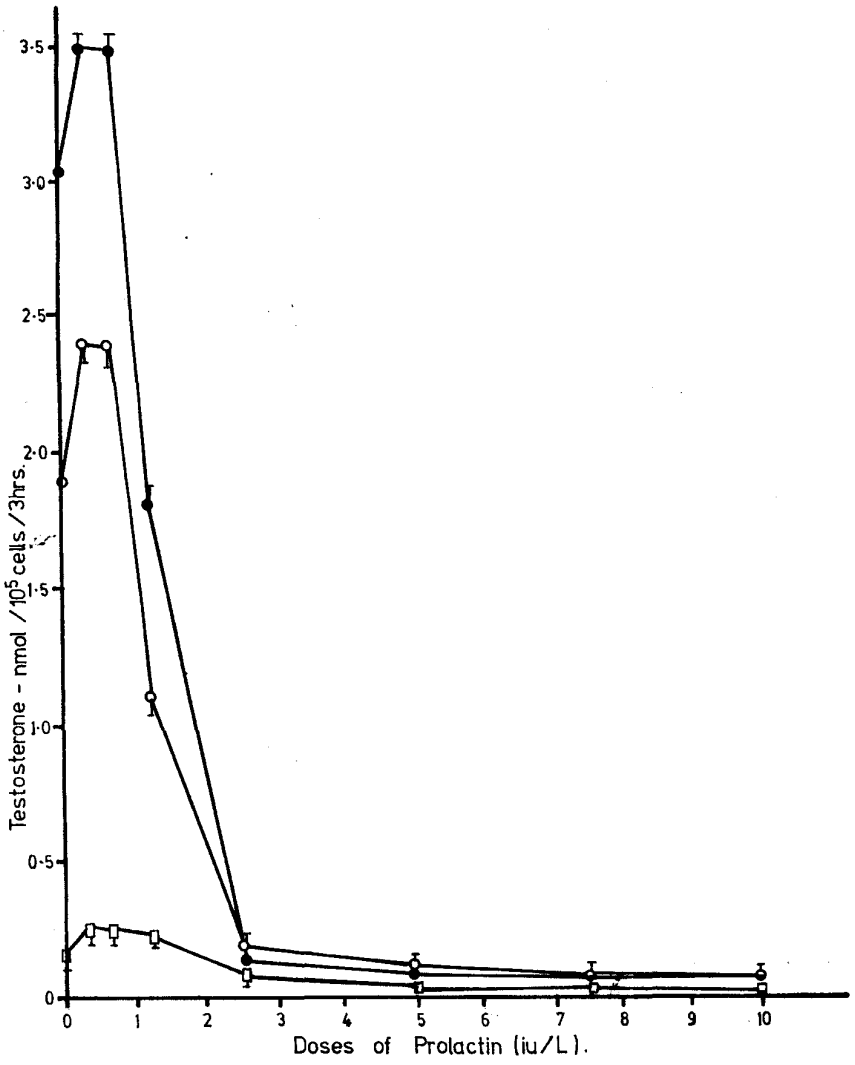

Fig 2
Effect of prolactin on Testosterone secretion in Leydig cells *in vitro*. □ = Prolactin alone; o =Prolactin + 10mM theophyline; • = Prolactin + LH

Fig. 2 shows the effect of graded doses of prolactin on testosterone secretion in Leydig cells in vitro. Testosterone secretions by 100, 000 Leydig cells in response to 0.31 and 0.62 i.u/L prolactin were significantly higher (P<).05) than the control values (0.01 nmol/10^5 Leydig cells/3hrs). Prolactin also significantly inhibited (P<0.001) in vitro Leydig cell steroidogenesis at doses of 1.25 – 10.0 i.u/L. Addition of 50ng/ml LH to the incubation medium had no effect on the inhibitory action of Prolactin (2.5 – 10.0 i.u/L) on testosterone secretion by Leydig cells. However, at lower doses of Prolactin, LH significantly stimualted testosterone secretion. Theophylline also did not produce any stimualtory effect on Prolactin-inhibited testosterone secretion (2.5 – 10.0 i.u/L Prolactin) in vitro. Moreover, at low doses of Prolactin, 10mM theophylline significantly increased (P<0.001) testosterone secretion, although lower that by LH (fig 2).

In most cases the final percentage Leydig cell viability (91.1 ± 2.7) when the

highest dose of Prolactin was administered did not significantly change from the initial percentage Leydig cell viability (94.1 ± 2.7) before hormone or drug administration.

DISCUSSION

One of the standardization procedures of the experimental model adopted in this study was the effect of LH on Leydig cell steroidogenesis *in vitro*. The significant increase in LH- induced testosterone secretion by these cells agrees with earlier reports that LH stimulates testosterone secretion *in vitro* (Sairam, 1978; Dafau, 1988). The significant rise in testosterone when theophylline was also present in the incubation medium further add to the existing evidence that LH stimulation of testosterone is via cyclic adenosine monophosphate (cAMP) second messenger system.

Marked decreases in testosterone secretion from Leydig cells were observed at higher doses of Prolactin (fig 2). However, at lower doses, a slight but insignificant increase in testosterone production in the presence of LH or theophylline was recorded at lower doses of Prolactin. These stimulants (LH and theophylline) had no effect on the inhibitory action of Prolactin on Leydig cell steroidogenesis at higher doses of Prolactin (fig. 1). These results confirmed that Prolaactin potentiated LH-stimulated testosterone secretion and provide evidence that at high blood Prolactin levels the male loses reproductive capability (Ganong, 1991). The sterility induced by hyperprolactinemia appears to be due to inhibition of testosterone secretion by Leydig cells *in vitro*. These workers employed high doses of Prolactin (50 – 1000ng/ml) which could cause receptor of desensitization and thus apparent loss of activity.

The significant rise in testosterone secretion by the Leydig cell *in vitro* in the presence of LH and theophylline in this study provide evidence that prolactin, like most other peptide hormones, may act via the cAMP second messenger system. The inhibition of Leydig cell steroidogenesis by high doses of prolactin justifies the claim of Thorner and Besser (1978) that hypoprolactinemia is usually associated with male infertility.

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