EFFECT OF BETA ADRENERGIC ANTAGONIST ON THE PRODUCTION OF TESTOSTERONE BY RAT'S LEYDIG CELLS

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Background: The Leydig cell is the source of the male sex steroids, or androgens, which are essential for the maintenance of the male phenotype, the male gonads, and spermatogenesis. It has been reported that patients taking beta-blockers experience sexual dysfunction. The purpose of this study was to explore the direct mechanism by which beta adrenergic antagonist exert its' effect on testosterone production by rat Leydig cells. **Methods:** Enzymatic dispersion of rat Leydig cell was done. About 85000 cells per tube were taken. After removal of endogenous testosterone by pre-incubation, The rat Leydig cells were incubated with varying concentrations of Atenolol: [Selective Beta-Adrenergic Antagonist} (10^{-6}, 10^{-7} and 10^{-9} M) with or with out LH 250 IU for three hours to measure the testosterone release by RIA. **Results:** Atenolol, in varying concentrations caused a significant ($P<0.05$) reduction in testosterone release by the rat Leydig cells as compared to the basal release of testosterone in a dose-dependent fashion. Atenolol decreased the testosterone release by LH stimulated Leydig cells more significantly ($P<0.001$) as compared to the effects of Atenolol produced on non-stimulated Leydig cells. **Conclusion:** The current data indirectly suggest that Atenolol inhibits testosterone releasee via mechanism involving decrease production of cAMP but not affecting the enzyme activities of steroidogenesis.

Key Words: Beta adrenergic antagonist; Atenolol; Leydig Cells; Testosterone

INTRODUCTION

The function of Leydig cells of the testis is to secrete androgens in a regulated fashion. The principal regulating mechanism consists of the secretion of pulses of luteinizing hormone (LH) by the adenohypophysis[1,2].It has long been known that cyclic AMP accelerates the synthesis of androgens by Leydig cells[3] and that LH increases the levels of cyclic AMP in these cells[4]. In view of the extensive evidence that cyclic AMP mediates the actions of various hormones, it has been concluded that the responses of Leydig cells to LH and hCG result from increased production of cyclic AMP as the result of the binding of LH to its receptor[5].

β-Adrenergic receptor antagonists (β blockers) have received enormous clinical attention because of their efficacy in the treatment of hypertension, ischemic heart disease, and certain arrhythmias. Ahlquist's hypothesis that the effects of catecholamines were mediated by activation of distinct α- and β-adrenergic receptors provided the initial impetus for the synthesis and pharmacological evaluation of β-adrenergic blocking agents[6]. Atenolol (Tenormin) is a β1-selective antagonist that is devoid of intrinsic sympathomimetic activity. Atenolol is very hydrophilic and appears to penetrate the brain only to a limited extent. Its half-life is somewhat longer than that of metoprolol[7].

Along with increasing use of various β-1- selective adrenergic antagonists in medical practice, a growing number of reports have emphasized the risk of sexual side effects[8]. Expanding information concerning their effect on adult male fertility will be of benefit to physicians, investigators and their patients.

MATERIAL AND METHODS

Two Wistar male rats, weighing 190± 10 g about 90 days old were taken per experiment. These rats were bred at animal house of Aga Khan University Karachi under standard conditions with a daily photo period of 16 hours light: 08 hours dark at 23°C. The rats had free access to food and water ad libitum.

The testes were dissected out and decapsulated. After decapsulation 4 testes were put in 10 ml of Eagles Medium 199 containing 0.25-mg/ml collagenase. This was incubated at 37° C for 25 minutes with a constant shaking at 100 cycles/minute in long axis parallel to the direction of the movement. 20 ml of cold saline was added to stop incubation process. Filtered portion was centrifuged at 80 g for 10 min at 4° C to remove collagenase.

The cell suspension was preincubated for an hour to remove any endogenous production of testosterone at 34°C. After pre-incubation, cell suspension was centrifuged for 10 min at about 200 g. The pellet was resuspended in incubation medium to give 85,000 viable cells per 200 μl [9].

Equal amounts of both Trypan blue (0.1%) and cell suspension were taken (30 μl each) for cell counting in Neubauer Chamber (WBC) (73). At least 85000 cells/200 μl were taken.

The rat Leydig cells were incubated with varying concentrations of Atenolol: [Selective Beta-Adrenergic Antagonist} (10^{-6}, 10^{-7} and 10^{-9} M) with or with out LH 250 IU for three hours to measure the testosterone release.

Testosterone was measured by radioimmunoassay (RIA) according to a WHO protocol, and regents were supplied through the WHO Matched Regent Programme. Testosterone was measured in extracted samples, whereas RIA reagents were directly added to tubes containing incubation medium without application of any extraction procedure. After addition of all the reagents, tubes were incubated overnight (28-24 h) and the bound fraction was separated from the unbound by the addition of 1% Dextran coated charcoal. Testosterone concentrations were calculated by logit-log transformation using a computer programme.

Statistical Package for Social Sciences Version 7.5 (SPSS 7.5) analyzed the data. The differences between the control and the treated samples were calculated by Student's "t" test. The arithmetic means and standard deviation were calculated for both samples separately.

The Confidence Interval was 95%. The values were considered significant when $P<0.05$ whilst these were labeled non significant when $P>0.05$ as compared to the levels observed in the control group.

RESULTS

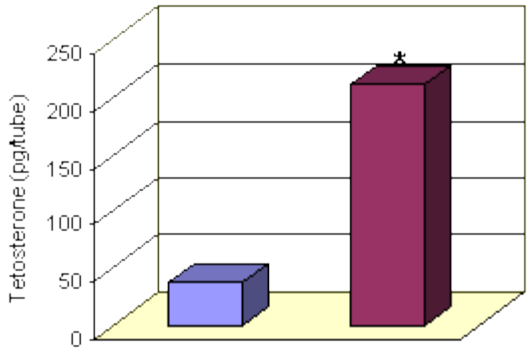
The basal release of Testosterone release was 37.15±0.25 pg per tube Administration of LH 250 IU increased the basal release of Testosterone significantly ($P<0.001$) which mounted to 211.43±6.62 pg/tube.(Fig 1)

Release of Testosterone under the effect of Atenolol 10^{-6} was 33.76±0.25 pg per tube, which was statistically significant ($P<0.05$) decrease as compared to basal release of 37.15±0.25 pg per tube. Atenolol at concentrations of 10^{-7} and 10^{-9}also did not exhibit any significant effects on Testosterone release.(Fig 2)

Administration of LH 250 IU increased the basal release of Testosterone significantly ($P<0.001$) up to 211.43±6.62 pg per tube. When LH 250 IU and varying concentrations of Atenolol were administered together, it was observed that Atenolol 10^{-6} and 10^{-7} caused a significant decrease in Testosterone levels (120.64 ±0.35 and 155.09 ±2.20 pg per tube) as compared to the levels of testosterone produced alone by LH. ($P<0.01$). While Atenolol 10^{-9} concentration along with LH 250 IU was unable to have significant effect.(Fig 3)

DISCUSSION

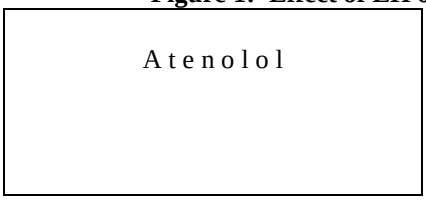
In the adult males, LH acts at multiple levels to stimulate steroidogenesis and to maintain normal Leydig cell function. In vitro, LH exerts immediate effects on protein synthesis, protein phosphorylation, and steroid synthesis, and has long-term effects on transcription of the steroidogenic enzymes and the intracellular structures important for steroidogenesis.[10]

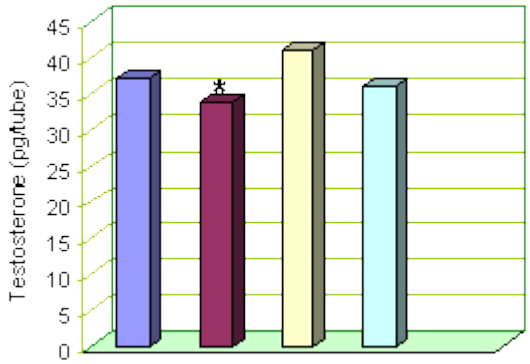


Control **LH 250 IU**

***LH 250 IU vs control** P<0.001

Figure 1: Effect of LH on Testosterone release by Leydig cells.





Control

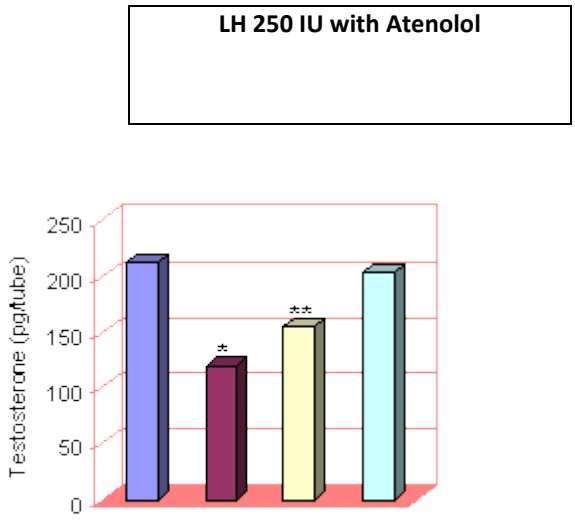
10^{-6} M 10^{-7} M 10^{-9} M

* Atenolol 10^{-6} vs control P<0.05

Fig-2: Effect of varying concentrations of Atenolol on Testosterone release by Leydig cells.

In the absence of LH in vivo, there is a rapid decline in Testosterone secretion by the Leydig cell and a gradual regression of the Leydig cells with loss of cytoplasmic volume and the intracellular structures associated with steroidogenesis, although Leydig cell numbers are marginally affected.[11]

In our study when Leydig cells were incubated with LH 250 IU for three hours it was observed that there was a significant (P<0.001) rise in the Testosterone release as compared to the basal release of Testosterone. The key steps of the steroidogenic pathway which are acutely regulated by LH action are the mobilization of stored cholesterol, transport of cholesterol into mitochondria and the resulting activity of the cholesterol side chain cleavage complex [2].



LH 250 IU **10^{-6} M** **10^{-7} M** **10^{-9} M**

***** LH with Atenolol 10^{-6} vs LH 250 IU P<0.001

****** LH with Atenolol 10^{-7} vs LH 250 IU P<0.01

Figure 3: Combined effect of LH and varying concentr-ations of Atenolol on Testosterone release by Leydig cells

It is well recognized that the administration of beta-blockers to patients after myocardial infarction improves their survival rate [12]. Review of previous usage of beta-blockers and of contraindications along with the current analysis of a uniform discharge summary has resulted in a significant increase in the use of beta-blockers as life saving drugs [13].

In the current study we observed that when Leydig cells were incubated with beta-1 selective antagonist: Atenolol, in a varying concentrations (10^{-6}, 10^{-7} & 10^{-9} M) caused significant (P<0.05) reduction in Testosterone release by the rat leydig cells as compared to the basal release of Testosterone in a dose-dependent fashion. This decrease in the Testosterone release by the Leydig cells under the effect of Atenolol seems to be the main cause of sexual dysfunction experienced by patients taking beta-blockers.

When Leydig cells were incubated with both LH 250 IU and varying concentrations of Atenolol (10^{-6}, 10^{-7} & 10^{-9} M), it was seen that the Testosterone release by the Leydig cells was significantly lower (P<0.001) as compared to the Testosterone release by the Leydig cells when incubated alone with LH 250 IU, again in a dose-dependent manner. Atenolol decreased the Testosterone release by LH-stimulated Leydig cells more significantly as compared to the effects of Atenololproduced on non-stimulated Leydig cells.

In a study Forgari et al has reported that Atenolol induces worsening of sexual activity and reduction of testosterone in hypertensive patients [14].

It is concluded that Atenolol causes a reduction in Testosterone release in a dose-dependent fashion both in non-stimulated and LH stimulated rat Leydig cells.

REFERENCES

1. Dufau ML, Veldhuis J, Fraioli F, Johnson MH, Catt KJ. Mode of bioactive LH secretion in man. J Clin Endocrinol Metab 1983; 57:93-1003.

2. Hedger MP, de-Kretser-DM. Leydig cell function and its regulation. Results-Probl-Cell-Differ 2000; 28 :69-110.

3. Sandler R, Hall PF. The influence of age upon the response of rat Biophys Acta 1968; 164:445-51.

4. Mellon SH, Vaisse C. cAMP regulates P-450scc gene expression by a cycloheximide insensitive mechanism in cultured mouse Leydig MA-10 cells. Proc Natl Acad Sci USA 1989; 86:7775-9.

5. Yano-K. The functional analysis of LH receptor. Nippon-Rinsho. 1997; 55 (2): 487-90.

6. Pujet JC, Dubreuil C, Fleury B, Proviedier O, Abella ML. Effects of celiprolol, a cardioselective beta blocker, on respiratory function in asthmatic patients. Eur. Respir. J 1992; 5:196-200.

7. Brodde OE. The functional importance of beta1 and beta2 adrenoceptors in the human heart. Am J Cardiol. 1988; 62:24C-29C.

8. Wadworth AN, Murdoch D , Brogden RN. Atenolol. A reappraisal of its pharmacological properties and therapeutic use in cardiovascular disorders. Drugs 1991:42:468-510.

9. Abdul Saeed S, Zaidi AA , Pertani SA. Effect of chronic treatment with cyclooxygenase inhibitor (indomethacin) on the pituitary-testicular axis. Med Sci Res; 1995: 23: 85-7.

10. Dufau ML, Miyagawa TF, Takada S, Khanun A, Miyagawa H, Boczko E. Regulation of androgen synthesis: the late steroidogenic pathway. Steroids 1997;62:128-32.

11. Duckett RJ, Hedger MP, McLachlan RI, Wreford NG. The effects of gonadotrophin-releasing hormone-immunization and recombinant follicle-stimulating hormone on Leydig cell and macrophage populations of the adult rat testis. J Androl 1997;18:417-23.

12. Kizer KW, Sawin CT. Increased use of beta-blockers after acute myocardial infarction. Am J Med 2000; 108(4): 349-50.

13. Dall L, Simmons T, Peterson S, Herndon B. Beta-blocker use in patients with acute myocardial infarction treated by hospitalists. Manag Care Interface 2000; 13(5): 61-3.

14. Fogari R, Preti P, Derosa G, Marasi G, Zoppi A, Rinaldi A, Mugellani A. Effect of antihypertensive treatment with valsartan or Atenolol on sexual activity and plasma testosterone in hypertensive men. Eur J Clin Pharmacol 2002;58 (3): 177-80.

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