# EFFECT OF PROPRANOLOL ON PROLACTIN IN ADULT MALE RATS

**Abdul Khaliq Naveed, *Liaqat Ali, Liaqat Ali Minhas, **Muhammad Tahir**

Army Medical College Rawalpindi, *Rawalpindi Medical College Rawalpindi, **University of Health Sciences Lahore

## ABSTRACT

***Objectives:*** The study was aimed to observe the effect on blood serum level of prolactin in propranolol treated adult male rats.

***Design:*** A experimental study.

***Place and Duration of Study:*** The study was done at Army Medical College Rawalpindi, National Institute of Health Sciences and NORI, Islamabad from Jan to Aug 2005.

***Materials and Methods:*** Adult male rats were used in the study. Rats were divided into four groups A (Control) and B, C and D (Treatment) each containing fourteen rats. Propranolol 1 mg, 2 mg and 4 mg/kg body weight intraperitoneally daily for sixty days was given to treated groups respectively and control group with 0.5 ml of distilled water. The blood serum level of prolactin was measured in half of the rats of all groups by Enzyme Immunoassay Method. Half of the rats were left for recovery to take place for another sixty days. The above procedure was adopted for the measurement of the prolactin level in the recovered rats. One way ANOVA was used to analyze the data.

***Results:*** The prolactin level in control and treated groups rat were 4.75 ± 1.4 ng/ml serum, 4.70 ± 1.1 ng/ml serum, 4.51 ± 1.7 ng/ml serum and 3.1 ± 0.8 ng/ml serum respectively. Prolactin level was significantly low ($p < 0.05$) in 4 mg treated groups when compared with control, treated 1 mg and 2 mg groups. After recovery period of sixty days insignificant difference in prolactin level between control and recovered groups was observed.

***Conclusion:*** Pattern of low prolactin level was observed following propranolol treatment in male rats.

**Keywords:** Adrenergic receptors, pituitary, prolactin

## INTRODUCTION

High blood pressure preventing drug like calcium channel blockers appears to affect the ability of sperm to bind and fertilize the egg. β-adrenergic blocking drugs are more commonly used by men (59%) than women (45.3%) because of high blood pressure and ischemic heart disease.

Retarted growth rate was observed following chronic oral propranolol treatment but it was independent of changes in plasma growth hormone a hypothalamic somatostatic concentration [1] Pituitary cells that were cultured when perfused with isoproterenol (a β-adrenergic agent) stimulated the prolactin release and was blocked by β-adrenergic antagonist [2] while the former effect was not observed on the corticotrophs [3] and gonadotrophs [4]. Adrenaline produces non-significant stimulatory effect on thyrotrophs [5].

One of the factors that control the expression of luteinizing hormone receptors

**Correspondence:** Brig Abdul Khaliq Naveed, Head Dept of Biochemistry & Molecular Biology, Army Medical College, Rawalpindi.
E-mail: khaliqnaveed2001@yahoo.com
khaliqnaveed2006@hotmail.com

on the Leydig cells is the prolactin [6] and low level of prolactin decreases the expression of luteinizing hormone receptors on the Leydig cells that may hamper the function of Leydig cells, one of the important factors that affect the male fertility.

The release of prolactin from pituitary is controlled by prolactin-releasing inhibiting factor by hypothalamus [7, 8]. During stress and breast suckling the prolactin release is regulated by prolactin-releasing factors from the hypothalamus [9-11]. Ascorbic acid prevents the oxidation of dopamine, one of catecholamine and potentiate the dopamine's inhibition on prolactin release at physiological concentration [12, 13].

A proportion of (20%) prolactin negative male are infertile, however this is not due to a defect in mating behavior [14]. Prolactin does play a role in male reproductive neuro-endocrine function by controlling the release of luteinizing hormone from anterior pituitary [15]. Although the prolactin negative male are not infertile completely but are 10% infertile. Those that are fertile only have a 40% chance of producing successful first pregnancy [16]. It is well known that catecholamine, such as epinephrine stimulates the multiple types of catecholamine receptors, it stimulates both α and β adrenergic receptors that decrease the peripheral vascular resistance at low concentration by activating the more sensitive β-receptors. Higher level of epinephrine excites the less sensitive α-receptors that increase the peripheral resistance. Raised levels of dopamine inhibit the prolactin release that was not statistically significant but at low concentration it significantly stimulates the prolactin release [17,18]. The stimulatory action of dopamine suggests the presence of highly sensitive catecholaminergic receptors [19].

Prolactin is released in the presence of phenoxybenzamine that inactivates the both α [20] and $D_2$ [21] but save the β-receptor [22] and isoproterenol which stimulated the β-receptors on isolated pituitary cultured cells suggests the involvement of β-receptors in vitro study.

Therefore the purpose of this in vivo study was to evaluate the effects of propranolol (non-selective β-receptor antagonist) on the adult male rat regarding the release of prolactin.

## MATERIALS AND METHODS

Fifty six adult male Sprague-Dawley rats were used in a quasi-experimental study, carried out at NIH and NORI Islamabad from Jan to Aug 2005. Animals were kept at average temperature of $25^0$C, with 10hr light and 14hr dark, fed on rat diet and water at libitum. Rat weight was 300-400gm and aged 3-4 months. Rats were divided into four groups A, B, C and D each containing 14 animals. These groups were further subdivided containing 7 rats in each sub-group. Sub-group $A_1$ experimental control, sub-groups $B_1$, $C_1$ and $D_2$ experimental, sub-group $A_2$ experimental recovery control and sub-groups $B_2$, $C_2$ and $D_2$ as experimental recovery. Group A was given 0.5ml of distilled water $kg^{-1}$ body weight and experimental group B, C and D were given 1mg, 2gm and 4mg. propranolol kg body weight intraperitoneally daily for sixty days. After 60 days treatment half of the animals from each group was kept for recovery and other half was sacrificed, 5ml blood collected from heart, allowed to clot in a tube and centrifuged in the same tube at 3000 rpm for 15 minutes. Supernatant was collected in polythene plastic tube and stored at $-20^0$C till the assessment of hormone level. The prolactin level was assayed by Enzyme Immunoassay Method, the diagnostic kit was Rat Prolactin EIA made by American Laboratory Products Company catalog number 12 MKVRP1. The lower limit of detection was 0.6ng $ml^{-1}$ blood serum. The intra-assay coefficient of variation was 3.9%. Half of the animals that were left for recovery to takes place for the period of sixty days the

same above procedure was repeated for blood sample collection and analysis of prolactin.

SPSS ver-13.0 was used to analyze the data. Mean +/- SD was used to describe the data. One way ANOVA and LSD post-hock test was used to compare the data.

## RESULTS

Weight of rats after 1, 2 and 4 mg propranolol intraperitoneal treatment and after recovery period each of 60 days was similar when compared with vehicle treated control group. Prolactin level in treated group $A_1$ control was 4.75 ±1.4ng $ml^{-1}$ serum and treated groups $B_1$, $C_1$ and $D_1$ was 4.7 ±1.1ng $ml^{-1}$ serum, 4.51 ±1.7ng $ml^{-1}$ serum and 3.15 ±0.8ng $ml^{-1}$ serum (table-1). Prolactin level in treated group $D_1$ was significantly low ($p < 0.05$) after sixty days treatment when compared with control group but there was insignificant low blood serum level of prolactin in group $B_1$ and $C_1$ rats when compared with control. The blood serum level of prolactin was compared among the treated groups this was insignificantly low between $B_1$ vs $C_1$ groups and significant low between $B_1$ vs $D_1$ and $C_1$ vs $D_1$ (table-2). After recovery period of sixty days no significant difference in blood serum level of prolactin between control and treated groups was observed (table-3).

## DISCUSSION

Releasing and inhibitory factors produced by the hypothalamus are peptides where as dopamine is catecholamine. The catecholamine receptors can stimulate different type of α, β and dopaminergic receptors.

Dopaminergic receptors on lactotrophic cell of pituitary in rat are negatively linked to the adenylyl cyclase system activity through GTP binding of the G1 subunit of G-protein. The β-receptors of the lactotrophic cells are positively linked when these receptors are stimulated and increase the secretion of these cells through G-subunits [23, 24] and non specific β-receptors agonists are positively linked and when the β-receptors are blocked by antagonistic drugs they show negative linkage with the lactotrophic cells of pituitary suggesting one of the antagonistic actions of the propranolol on pituitary lactotrophs in this study.

Since activation of cyclic AMP stimulates the prolactin release [25] and study on the β-receptors blockage show a decreased prolactin release. So our conclusion indirectly is consistent with the concept that blockage of β-receptors action inhibit the adenyl cyclase system. Since epinephrine contribute a stimulation of prolactin release during stress as hypophyseal blood has high level of epinephrine concentration [26] show coupling of stress-induced prolactin release. So indirectly it proves that the lactotrophic cells stimulation is related to adrenergic receptors that work by the activation of cyclic AMP and propranolol decreases the stimulation of prolactin release by blocking the cyclic AMP. Staining pattern of adenohypophysis mammotrophic cells was not weak in pain inflicted rabbits as compared to the diffuse traumatic brain injured rabbits postulating that pain alone may not be important factor in the hormonal response to trauma [27] or pain of giving an injection, similarly may not have affected the prolactin level in the vehicle/ propranolol injected rats in the current study. Intracerebroventricular administration of prolactin releasing peptide (RFRP-1 in the 3 nmol concentration, potent mediator of stress response in brain) significantly elevated the prolactin release in conscious male rats but prevented the stimulation of prolactin secretion when pretreated with catecholamine synthesis inhibitor, alpha-methyl-para-tyrosine [28] whether the concentration of releasing peptide achieved by the stress of injection in the control rats affected the prolactin level is debatable and need to be explored. Result showed significantly decreased plasma cortisol, prolactin and TSH levels in chronic post-traumatic stress disorder patients [29]. Whether the stress of intrperitoneal injection was up to the extent that affected the prolactin level in experimental rats was not determined by psychometric instruments. Further study is required in this regard.

**Table-1: Serum prolactin level (ng/ml) after 60 days treatment.**

| Groups | n | Mean ± S.D |
|---|---|---|
| $A_1$ | 07 | 4.75 ± 1.40 |
| $B_1$ | 07 | 4.70 ± 1.10 |
| $C_1$ | 07 | 4.51± 1.71 |
| $D_1$ | 07 | 3.15 ± 0.83 |

**Table-2: Comparison of mean level of serum prolactin between various groups after treatment.**

| Groups | P-value | Level of significance |
|---|---|---|
| $A_1$ VS $B_1$ | > 0.05 | N. S.* |
| $A_1$ VS $C_1$ | > 0.05 | N. S.* |
| $A_1$ VS $D_1$ | < 0.05 | S.** |
| $B_1$ VS $C_1$ | > 0.05 | N. S.* |
| $B_1$ VS $D_1$ | < 0.05 | S** |
| $C_1$ VS $D_1$ | < 0.05 | S** |

**Non significant, ** Significant*

**Table-3: Serum prolactin level (ng/ml) after 60 days recovery.**

| Groups | n | Mean ± S.D |
|---|---|---|
| $A_2$ | 07 | 6.3 ± 1.8 |
| $B_2$ | 07 | 5.9 ± 1.6 |
| $C_2$ | 07 | 8.1± 3.1 |
| $D_2$ | 07 | 5.3 ± 3.0 |

β-receptors stimulant isoproterenol effectively enhances the prolactin release from primary cultured pituitary lactotrophic cells [2]. Several other studies have proved and support the role of prolactin release indirectly through central axis [30,31] but much evidence also exists about the release of more prolactin by the pituitary without involving the higher level of the rat [32,33]. Whereas the β-adrenergic agonistic drugs failed to stimulate the prolactin release from the freshly cut isolated lamb pituitary cells [34] which are contrary to the observation made in this study it may be due to differences in species or cultured growth environment of pituitary cells when compared with this study.

It was observed that propranolol block the prolactin release in pituitary cells which is in agreement to the study by Seon et al [2] in which cultured pituitary cell with β-receptors agonist stimulated the prolactin release not only by the paracrine action, but also due to the presence of β-receptors in pituitary lactotrophs which are coupled to block the cyclic AMP stimulation system that is in consensus to the observation made in this study.

Prolactin receptor knockout male mice showed infertility in 10% of male mice and only have 40% chance of producing successful first pregnancy [16]. Suzuki et al [35] found in patients taking atenolol demonstrated sexual dysfunction and mild reduction in serum testosterone concentration which mimic to this study in which after propranolol treatment there was significant low level of serum prolactin possibly may adversely affect the male fertility.

## CONCLUSION

Conclusively adult male rat showed reduction in blood serum prolactin concentration following intraperitoneal propranolol treatment.

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