Influence of Neurotransmitters on GnRH release in Letrozole induced Polycystic Ovarian syndrome rat model

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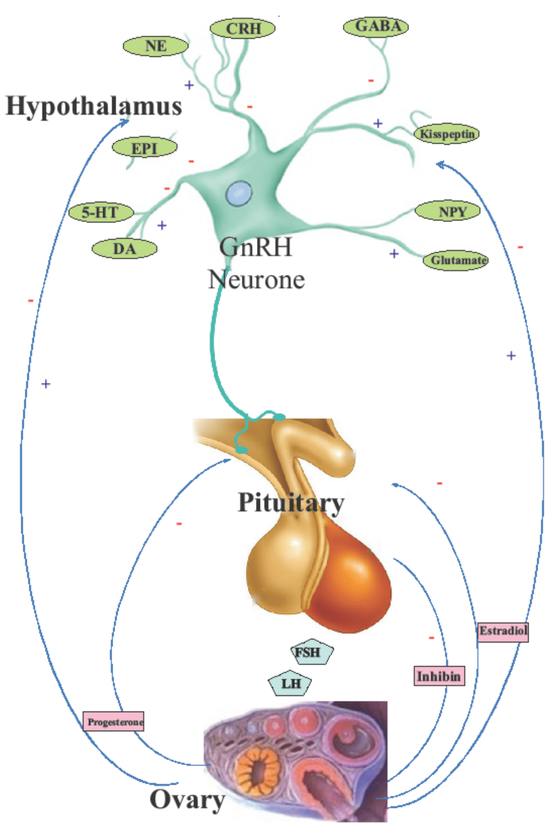
Introduction

- The reproductive function of the body is under the control of Hypothalamic-Pituitary-Ovarian (HPO) axis.
- At the brain level, control of reproduction is governed by cumulative interactions of several intrinsic and extrinsic factors. These factors include neurotransmitters which directly or indirectly regulate HPO axis.
- Any alteration in HPO axis may lead to reproductive abnormalities such as the Polycystic Ovarian Syndrome(PCOS).
- PCOS is a complex endocrine disorder affecting 6-8% of females in reproductive age (Goodarzi and Azziz, 2006).
- A prominent feature of PCOS is increased pulsatility of the GnRH leading to rapid release of LH, resulting in elevated LH:FSH ratio (Diamanti-Kandarakis, 2008).This further results into increased androgen production, chronic anovulation and cyst formation in ovary (Franks *et al.*, 2008).
- Though hyperactivation of Hypothalamic-pituitary(H-P) axis has been well understood in PCOS phenotype, the clear link between the factors which control H-P axis remains unclear.
- *The aim of the current study is to understand the status of neurotransmitters and their receptors in PCOS condition using rodent model.*

Hypothalamic-Pituitary-Ovarian axis

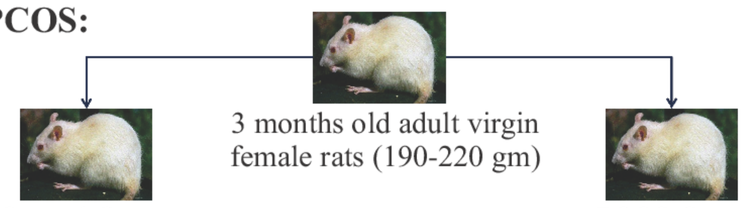


Methods

1. PCOS induction in rats by using Letrozole-an aromatase inhibitor (Kafali *et al.*, 2004)
3. Estrus cyclicity (Yener *et al.*, 2006)
4. Estimation of Neurotransmitters
   - -Serotonin, Dopamine, Norepinephrine (by HPLC-ECD)
   - - GABA (Lowe *et al.,* 1958)
5. Hormone analysis (Estrogen, Testosterone, progesterone) by ELISA kits
6. RNA isolated using TRIzol reagent was used for first strand cDNA synthesis and a Real-Time PCR analysis was carried out.

Experimental Design

Induction of PCOS:



Validation of PCOS rat model:

Table-1. Serum hormone profile

	Control	PCOS
Estrogen (pg/ml)	132.3 ± 17.37	139.3 ± 19.10
Testosterone (ng/ml)	0.3157 ± 0.055	1.177 ± 0.072 ***
Progesterone (ng/ml)	12.25 ± 0.54	9.95 ± 0.39 *

Table-1. Letrozole treated rats showed increased serum testosterone (P<0.001) compared to control group with decreased progesterone content (P<0.05) and no difference in serum estradiol level.

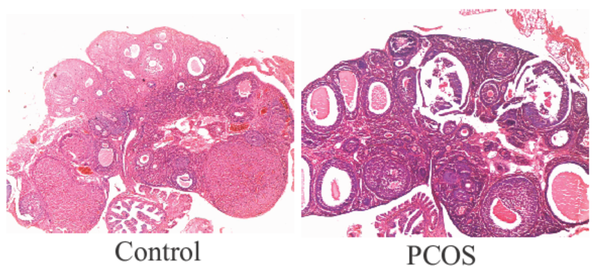


Figure 1.Hematoxylin-eosin-stained ovarian sections. Control sections show follicles in various stages of development. Treatment group sections demonstrate numerous peripheral fluid-filled cysts (4X).

Table-2. Estrus cycle profile

	Normal Cycle	Extended Proestrus	Extended Estrus	Extended Metestrus	Extended Diestrus
Control	100%	-	-	-	-
PCOS	-	5%	-	5%	90%

Results

1. Status of Gonadotropins

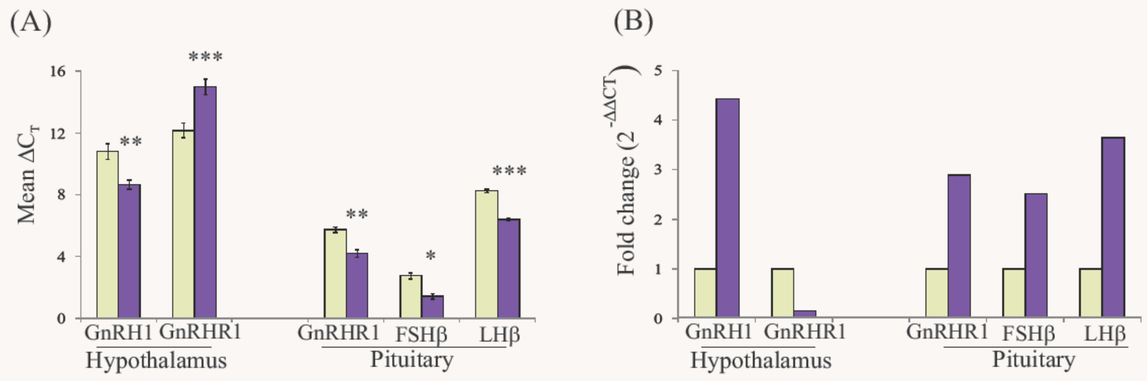


Figure-1: mRNA expression profile of Gonadotropins.
All values are presented as Mean±SEM; **P<0.01; *P<0.05 as compared to control

2. Estimation of Neurotransmitters

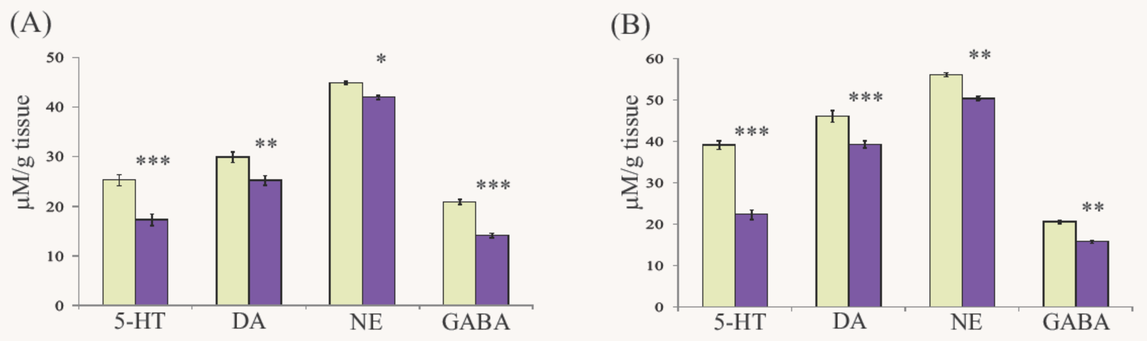


Figure-2: Neurotransmitter estimation from Pituitary (A) and Hypothalamus (B)
5-HT: Serotonin; DA: Dopamine; NE: Norepinephrine; GABA: γ-amino-butirc acid

3. Gene expression of Neurotransmitter receptors

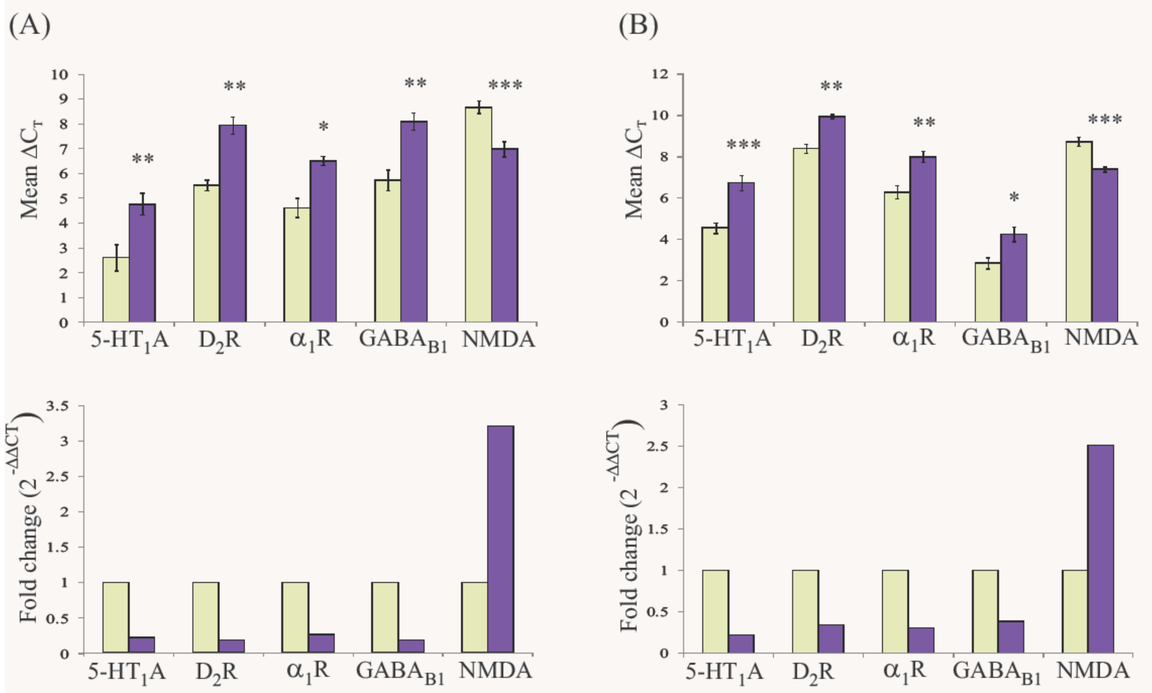


Figure-3: Real-Time PCR analysis of Neurotransmitter receptors in Pituitary (A) and Hypothalamus (B)
5-HT1A: Serotonin 5-HT1A receptor; D2R: Dopamine D2 receptor;
α_1R: alpha1-adrenergic receptor; GABAB1 receptor; NMDA: Glutamate NMDA receptor
***P<0.001; **P<0.01; *P<0.05 as compared to control group

Conclusion

- Letrozole treated rats exhibited an altered hormonal profile, disturbed estrus cyclicity and peripheral cysts in the ovary, suggesting that letrozole induced PCOS-like condition in rodents.
- Increased GnRH pulsatility has been established in PCOS phenotype. Present study shows increased transcript levels of GnRH1, GnRHR1, LH-β, FSH-β, which indicates increased gonadotropin production in PCOS condition. Decreased hypothalamic GnRHR transcript suggest reduced feedback signal to GnRH release.
- Neurotransmitters play a central role in the regulation of reproductive function, directly influencing GnRH release from hypothalamus (Szawka *et al.*, 2013).
- Reduction in the neurotransmitter levels with down-regulation of their receptor transcripts could result into decreased inhibitory signals to GnRH, leading to increased GnRH pulsatility in PCOS. Also, similar expression pattern of these neurotransmitters in pituitary indicates their interaction with gonadotropins, leading to an increased LH:FSH ratio.
- This study, for the first time, demonstrates that an alteration in regulatory molecules, like the neurotransmitters, affects GnRH pulsatility, resulting in the pathogenesis of PCOS.

References:

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