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 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 Azizi, 2006).
- A prominent feature of PCOS is increased pulsatility of the GnRH leading to rapid release of LH, resulting in elevated LH:FSH ratio (Diament-Kandaswaras, 2008). This further results into increased androgen production, chronic anovulation and cyst formation in ovary (Franks et al., 2006).
- Though hyperacivation of Hypothalamic-Pituitary-Ovarian (H-P-O) axis has been well understood in PCOS phenotypes, the clear link between the factors which control H-P-O 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.

Methods

1. PCOS induction in rats by using Letrozole-an aromatase inhibitor (Kafali et al., 2004)
2. Estrus cyclicity (Yener et al., 2006)
3. Estrus cyclicity (Yener et al., 2006)
4. Estimation of Neurotransmitters
   - Serotonin, Dopamine, Noradrenaline (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:

Control Group
(Carboxy methyl cellulose treated)

3 months old adult virgin female rats (190-220 gm)

PCOS Group
(0.5 mg/kg B.W. of Letrozole, orally, daily for 21 days)

Validation of PCOS rat model:

<table>
<thead>
<tr>
<th>Table 1. Serum hormone profile</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Estrogen (pg/ml)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
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<tr>
<td>Progesterone (ng/ml)</td>
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Table 2. Estrous cycle profile

<table>
<thead>
<tr>
<th>Control</th>
<th>PCOS</th>
</tr>
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<tbody>
<tr>
<td>Normal Cycle</td>
<td>100%</td>
</tr>
<tr>
<td>Extended Proestrus</td>
<td>-</td>
</tr>
<tr>
<td>Extended Estrus</td>
<td>-</td>
</tr>
<tr>
<td>Extended Metestrus</td>
<td>-</td>
</tr>
<tr>
<td>Extended Diestrus</td>
<td>-</td>
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</tbody>
</table>

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

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

Table 3. Gene expression of Neurotransmitter receptors

<table>
<thead>
<tr>
<th>Table 3. Gene expression of Neurotransmitter receptors (A)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>5-HT1A</td>
</tr>
<tr>
<td>5-HT1B</td>
</tr>
<tr>
<td>5-HT2A</td>
</tr>
<tr>
<td>5-HT2B</td>
</tr>
<tr>
<td>alpha(1)</td>
</tr>
<tr>
<td>alpha(2)</td>
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Results

1. Status of Gonadotropins

- mRNA expression profile of Gonadotropins. All values are presented as Mean(SEM); **p<0.01; *p<0.05 as compared to control group

2. Estimation of Neurotransmitters

- Neurotransmitter estimation from Pituitary (A) and Hypothalamus (B)

3. Gene expression of Neurotransmitter receptors

- Real-time PCR analysis of Neurotransmitter receptors in Pituitary (A) and Hypothalamus (B)

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, GnRH1, LH, FSH-β, which indicates increased gonadotropin production in PCOS condition. Decreased hypothalamic GnRH 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 (Stawka 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:

- Lowa EPJ, El, Eyram SS. (1958) Neurochem. 3:8-1