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Dexamethasone Induced Modulation of Reproduction: Investigating Role of Dexras1 Monomeric G Protein

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Abstract
Many factors like hormonal, anatomical, genetic, and environmental etc effect reproduction. Stressful conditions like emotional-stress, altered level of hormones etc can cause alterations in release of reproductive hormones thus ultimately effecting reproduction. In our study, we have focused to know the modulation of reproduction induced by Dexamethasone (DEX) (a synthetic glucocorticoid), which mimics as a stress condition in female mice and investigated the role of Dexras1 in reproduction under the influence of Dexamethasone. Glucocorticoid are known to influence the release of GnRH hormone which in turn regulate the release of reproductive hormones (LH, FSH). Dexamethasone is reported to upregulates Dexras1 expression. So we have investigated the modulation of reproduction by alteration in the release of reproductive hormones LH and FSH under DEX dosage in female mice. We observed that the level of hormones LH and FSH altered, in the presence of Dexamethasone doses (0.1 and 0.5mg/kg mice). LH level is decreased while the level of FSH increased with DEX dose. So the present study reveals the role of Dexamethasone in modulation of reproduction. It has been reported earlier that Dexras1 is upregulated by Dexamethasone so we could suggest that Dexras1 might involve in modulation of reproduction and could become a very important therapeutic target in reproduction signaling processes.

Keywords- Reproduction, Dexamethasone, Dexras1, Reproduction, Stress, LH, FSH, Glucocorticoids.

1. Introduction

In many studies it has been reported that Glucocorticoids regulates expression of gonadotropin hormones (GnIH & GnRH) which influence the release of FSH and LH hormones. One of the main causes for reproductive cycle modulation is the “stress” condition. Stress can suppress the activity of reproductive neuroendocrine axis. In some species elevation in glucocorticoid mimic the effects of stress, as reported in mice (Breen et al., 2012). The elevated glucocorticoids diminish the ability of pituitary to respond to GnRH in female mice (Breen and Mellon, 2014). Glucocorticoids also alter the function of pituitary to express genes. It acts directly upon anterior pituitary gonadotrope cell to suppress GnRH induction of LHβ gene expression (Breen et al., 2012). The level of LH and FSH alters during surgical stress in mice (Dardes et al., 2000).

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In the present study, we are focusing on Dexamethasone induced modulation of reproduction physiology under stress conditions and investigated the role of Dexras1 GTPase in reproductive signalling processes. Dexras1 is a monomeric G protein which is reported to be involved in modulation of various physiological processes but, the role of Dexras1 and small GTPases in pathways involved in synchronization and molecular signaling mechanism(s) in reproduction is still not known. Dexras1 is rapidly induced and upregualted by dexamethasone (a synthetic glucocorticoid) (Kemppainen and Behrend, 1998). The expression of Dexras1 under the influence of Dexamethasone was reported in central nervous system and spinal cord transaction of rat (Li et al., 2008; Gao et al., 2010). Dexamethasone is reported to influence the release of GnRH hormones (Gore et al., 2006; Soga et al., 2012; Gooyandeet al., 2014) which itself responsible for the expression of FSH and LH type reproductive hormones. In recent studies, high expression of Dexras1 was reported in reproductive tissue like uterus (in mouse), ovary and testis. Dexras1 reported to be a novel signal transducer in female reproductive organs. Its expression increased by estradiol but not by progesterone (Kim et al., 2017). Recently Dexras1 role in uterus implantation failure has been reported (Hong and Choi, 2018). Its expression was down regulated in the uterine endometrium of repeated implantation failure.

We hypothesize that Dexras1 monomeric G protein might be involved in modulation of reproduction under the influence of different hormones as a synthetic hormone Dexamethasone. We approached towards the in vivo experimentation on animals (female mice) to analyze alteration in reproductive hormones by dexamethasone (synthetic glucocorticoid) treatment.

2. Materials and Methods

2.1 Animal Acclimatization

Animals, Swiss albino female mice were acclimatized to laboratory conditions. Animals were kept under controlled conditions of light and temp, and were free access to food and water and *ad libitum*. Female mice were synchronized in groups before examine. 30-40 days old, nine animals were grouped on the basis of DEX treatment after taking animal ethical clearance (IAEC, SGRRITS, Dehradun, Uttarakhand).

2.2 Experiment Design

In order to observe the effect of Dexamethasone (DEX) doses and modulation of reproduction the experiments is designed to observe altered level of reproductive hormones under DEX treatment.

2.3 Dexamethasone Doses Administration

Tablets of Dexamethasone, 0.5 mg (Dexona), ZydusGeO (Zydus Healthcare Limited) was commercially available. The stock was prepared by dissolving 1 tablet of 0.5 mg in 2 ml distilled water. Group1 is kept as control (without any dose treatment), Group 2 and 3 were

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treated daily with 0.1 and 0.5 mg/kg/mice as described in table 1. Doses were decided as per protocol by (Schafer et al., 2005; Gooyande et al., 2014; Dolatabadi and Zarchii 2015) with slight modifications. The doses were administrated orally, using oral feeding needle (Figure 1) that yielded 0.1 and 0.5mg/kg body weight of female mice in different groups. The experiment was carried out for10 days. In order to observe any therapeutic effect, body weight, food intake, inactivity, and mortality were monitored daily in each group.

Table 1. Group-wise dosage of DEX

S. No.	Group of female mice (n=3 mice/group)	DEX dose/kgmice
1.	Group 1(Control group)	Without any dose
Experimental groups		
2.	Group2	0.1mg/kg
3.	Group 3	0.5mg/kg

Figure 1. Oral administration of DEX dose in mice

2.4 Collection of Blood Samples

Samples were collected as per ethical norms of animal house (SGRRITS, Dehradun). To measure the alteration in the release of reproductive hormone under the influence of Dexamethasone treatment, the sample of blood was collected from tail of mice and the serum sample was used for the hormonal profiling of two reproductive hormones: FSH and LH. The serum was obtained by centrifugation of blood at 4000 rpm for 20 min at 4^{o}C and was stored at -20^{o}C (Fernandez et al., 2010; Shirasaki et al., 2012) for further hormonal measurement.

2.5 Hormonal Profiling: Measurement of FSH and LH Hormones

The reproductive hormone profiling of two different hormones FSH, LH was done by ELISA method. For the analysis of reproductive hormone, the ELISA kits of Bio-Detect was used (LH ELISA cat. No 1011, FSH ELISA cat. No 1012). As per the kit manual, 50µl serum sample was loaded in each well and the reproductive hormonal level measured by ELISA reader (Alere AM microplate reader).

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3. Result and Discussion

The indicative values obtained showed the altered level of reproductive hormone under the influence of DEX as compared to control (table 2). The value of LH hormone decreased with increased dose of dexamethasone. The indicative level of LH hormone showed the average value 20.99ng/ml in control group. While, in case of experimental groups the indicative average values were observed13.87ng/ml and 13.07ng/ml with 0.1mg/kg and 0.5mg/kg DEX treated mice respectively (Figure 2). The level of hormone FSH was increased with increase in dosage. The indicative average value of FSH in control group was 0.05ng/ml. While in experimental groups these indicative values were 0.09ng/ml and 0.31ng/ml with 0.1mg/kg and 0.5 mg/kg in DEX treated mice respectively (Figure 3).

Table 2. Values of hormones obtained by ELISA assay

Hormone	Group1(control) Without any dose Values(ng/ml)	Group2 (0.1mg/kg)	Group3 (0.5mg/kg)
LH	21.41	14.28	13.29
	20.76	13.92	13.17
	20.82	13.41	12.76
Average value of LH	**20.99**	**13.87**	**13.07**
FSH	0.05	0.09	0.27
	0.03	0.09	0.30
	0.08	0.11	0.37
Average value of FSH	**0.05**	**0.09**	**0.31**

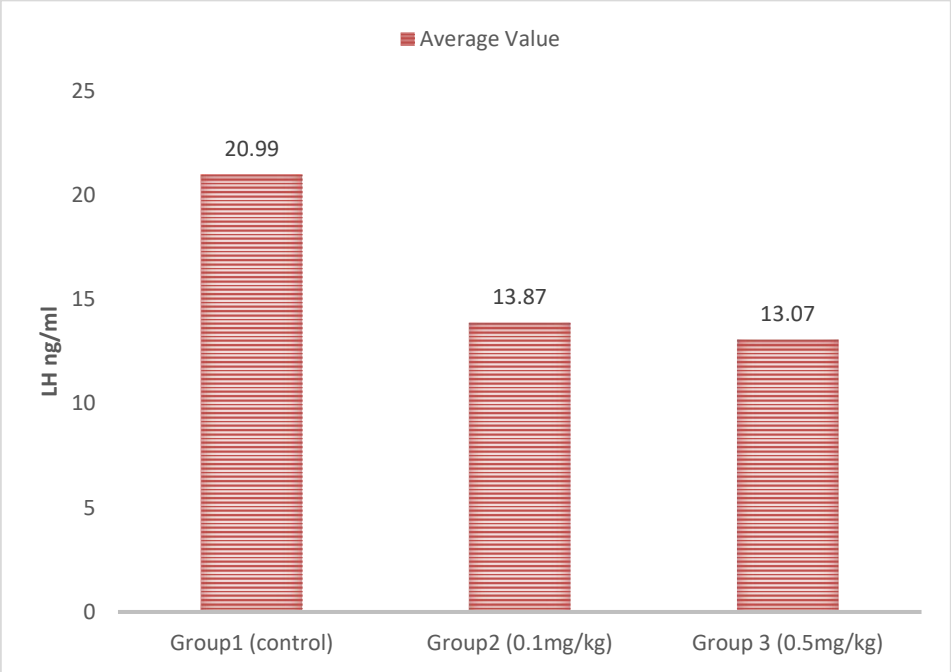

Figure 2. The level of LH hormone with DEX dosage

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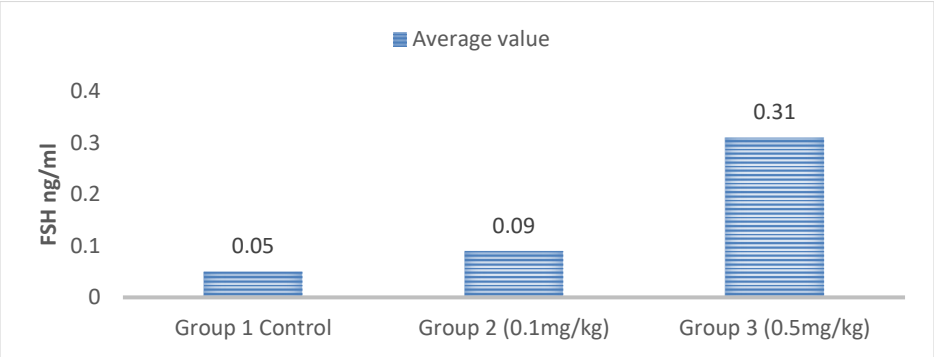

Figure 3. The level of FSH hormone with DEX dosage

4. Conclusion

The present study showed the effect of G protein Dexras1 in release of reproductive hormone and modulation of molecular signaling pathways of reproduction after DEX dose (which mimic the stress condition). Dexras1 is upregulated by Dexamethasone. The study suggested that Dexamethasone affect the release of reproductive hormones. The level of LH and FSH were altered under the influence of Dexamethasone. It has been studied that LH is a key hormone that is involved in ovulation. LH surge occurs for ovulation and both LH and FSH hormones alter during different stages of reproductive cycle. In the present study, it has been observed that due to DEX treatment the LH level decreased and FSH increased so this alteration effect the stages of reproductive cycle hence influence the reproductive cycle also. Our focus is on role of Dexras1 role in reproduction and this protein itself upregualted by Dexamethasone so these finding suggest that these alterations might involve the modulation by Dexras1. The findings might have therapeutic importance in concern to women reproductive health and infertility problems also.

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