

Adrenocortical Response to 17-Beta Estradiol Replacement in Oophorectomized Female Sprague Dawley Rats

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ABSTRACT

Objective: To determine the effect of estradiol treatment on serum corticosterone levels in Oophorectomized (OVX) female Sprague Dawley rats exposed to chronic restraint stress.

Study Design: Experimental study.

Place and Duration of Study: Department of Physiology, Army Medical College, Rawalpindi and National Institute of Health, Islamabad, from January to December 2008.

Methodology: A total of 90 female Sprague Dawley rats (age: 90 ± 10 days), were divided into three groups, each having 30 rats. Group-I comprised of healthy control female rats whereas group-II and III were experimental female rats exposed to chronic restraint stress after bilateral Oophorectomy and called estradiol treated and vehicle treated groups. Estradiol treatment of Oophorectomized rats was done once daily for 2 weeks. At the end of experiment, the rats were sacrificed and intracardiac blood sampling was done to measure serum corticosterone levels by enzyme linked immunosorbent assay (ELISA) kit.

Results: The restraint stress to estradiol treated rats for 2 weeks revealed that serum corticosterone levels were significantly increased (31.32 ± 5.46 ng/ml, $p < 0.05$) as compared to the healthy controls (17.48 ± 4.14 ng/ml).

Conclusion: Chronic restraint stress results increases the serum corticosterone levels in Oophorectomized Sprague Dawley rats. Estradiol treatment increases the responsiveness of adrenal cortex of Oophorectomized female rats.

Key Words: Stress. Hypothalamic-pituitary-adrenal (HPA) axis. Corticosterone. Estrogen. Oophorectomy.

INTRODUCTION

Stress is the specific response of the body to a stimulus that interferes or disturbs the normal physiological equilibrium of an organism.¹ It denotes the real or perceived perturbation to the organism's physiological homeostasis or psychological well-being. The stress response comprises of constellation of behavioural or physiological mechanisms to counter the perturbations to restore normalcy. These include behavioural reactions, activation of sympathetic nervous system, secretion of stress hormones (e.g. glucocorticoids) and mobilization of immune system. Activation of the hypothalamo-pituitary adrenal (HPA) axis is an essential adaptive mechanism that enables the human body to maintain physiological stability in response to stressful stimuli.²⁻⁴

Stress involves two-way communication between the brain and various systems of the body via neural and endocrine mechanisms.^{5,6} Most of the time, stress is not

harmful and damaging for the human body, especially in its acute form when it has protective effects. If stress runs a long-course, it almost always has deleterious and damaging effects on the individual, leading to serious health consequences. However, the actual precipitation of a particular disorder depends upon intensity and chronicity of stress on a specific body system. The age and gender of an individual is an important consideration regarding the prevalence of disease as exemplified by the increased incidence of cardiovascular diseases in middle aged men compared to age matched women.^{7,8}

The HPA axis constitutes the major regulatory axis that reacts to stress and release cortisol which helps to restore basal conditions prior to stress. The elevated levels of CRH, ACTH and cortisol are often used as indices of stress.⁹ Adult men and women have different physiological stress responses which is an important determinant of future health and disease outcome.

Compared with females of the same age, males of many species manifest higher incidence of arteriosclerosis and infections. An important cause of such gender differences in infections is the difference in the endocrine-immune interaction.^{10,11}

A number of studies have been done in the past to establish the variable stress response in males and females as an important predictor of their health status but their results are controversial. Gender influences the HPA axis hormonal response to stress, manifesting higher levels of ACTH and corticosterone in females

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while others show the reverse as true. However, in humans, no such definite gender differences have been established.

Although the impact of gender on hypothalamo-pituitary-adrenal axis response to stress largely remains inconclusive, yet the possible role of sex steroids on such sex specific differences could not be ignored. The female hormone, estrogen has been implicated to exert strong stimulatory influence on the optimal functioning of HPA axis with modulatory effects on glucocorticoid and mineral corticoid receptors but only very few experimental studies have investigated this vital role of estrogen.

The present study was, therefore, designed to determine the effect of estradiol administration on serum corticosterone levels in Oophorectomized female Sprague Dawley rats exposed to chronic restraint stress.

METHODOLOGY

This study was conducted at the Department of Physiology at Army Medical College in collaboration with National Institute of Health, Islamabad, from January to December 2008, after approval by the Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi.

Ninety healthy female Sprague Dawley rats with an average body weight of 200 ± 50 gm aged between 90 ± 10 days were included in this experimental study by convenience sampling. The rats were randomly divided into three groups by lottery method; each having 30 female rats. Group-I comprised of 30 healthy intact female rats not exposed to any kind of experimental stress. Group-II comprised of 30 female rats which underwent bilateral removal of ovaries; were injected with exogenous $17\text{-}\beta$ estradiol in a dose of $20 \mu\text{g}/\text{kg}$ body weight,¹² daily by subcutaneous injection and subjected to chronic restraint stress for 6 hours daily for the period of 14 days. The pharmacological doses of estradiol as replacement therapy in Oophorectomized rats are different in different studies. They depend on factors such as age of the Oophorectomized female rat, species of rat, its body weight, type and duration of exposure to stress. The stock solution was prepared by dissolving 250 mg of powdered estradiol in cotton seed oil. The stock solution was stored in vials within a desiccant filled plastic containers tightly closed with a lid.

Group-III consisted of 30 female rats that underwent surgery and were exposed to similar stress regimen as group-II but only cotton seed oil as a vehicle was injected for 14 days.

The exclusion criteria were male and diseased rats. For induction of anaesthesia, a large round glass container with a lid at the top and a piece of wire mesh at its floor

was used. Ether soaked cotton was placed in mesh over the chamber and it took 3 – 5 minutes to get the rat anaesthetized. For Oophorectomy, 1.5 – 2 cm midline dorsal incision was given on the skin with a sharp sterile surgical blade about half way between the middle of the back and base of the tail. Anterior abdominal muscles were excised, peritoneal cavity was exposed. Ovaries were identified as small ovoid granulated pinkish purple structure having the appearance of a mulberry. Sterile chromic 1 ligature on either side of the ovary and with the help of the sterilized artery forceps, the ovary was removed between the ligatures. For administration of chronic stress, 12 x 8 inches wooden board was taken. Two flexible metallic clips were fixed on either end of the board about 2 inches from each side with the help of screws. The flexibility of the metallic clips allowed the adjustment required during the placement of the experimental rat inside the restrainer. A plastic restrainer measuring about 8 x 9 inches, having a volume of 260 ml was used as a restrainer to expose experimental rats to restraint stress. The bottom of the restrainer was finely cut, leaving a small area intact so that its base could be closed when required. Several tiny holes were made in the restrainer cap for aeration and continuous oxygen supply during the experiment.

At the end of the study, after 2 weeks, rats in all groups were anaesthetized and intracardiac blood sampling was done. Serum separated after centrifugation at 5000 rpm for 5 minutes, stored at -80°C in Eppendorf tubes. Serum corticosterone levels in all the groups were measured by using ELISA technique.

Statistical analysis was done on Statistical Package for Social Sciences (SPSS) version 13.0. Mean and Standard deviation (SD) was calculated for quantitative variables. Change in serum corticosterone level between all groups were compared by using one-way ANOVA and Post-Hoc Tukey's test.

The level of significance was taken at 5% and p-value less than 0.05 was considered significant.

RESULTS

Serum corticosterone levels in female groups were statistically significant ($p < 0.05$) when analyzed by ANOVA as presented in Table I.

There was significant difference between mean levels of serum corticosterone levels amongst the individual groups of female rats, as mentioned in Table II.

Table I: Overall comparison of corticosterone levels between healthy control female rats and restraint stress exposed Oophorectomized female Sprague Dawley rats with and without administration of estradiol.

Parameter	Group-I	Group-II	Group-III	p-value
Serum corticosterone (ng/ml)	17.48 ± 4.14	31.32 ± 5.46	24.85 ± 5.49	< 0.05

Table II: Comparison of serum corticosterone levels between healthy control female rats and restraint stress exposed Oophorectomized female Sprague Dawley rats with and without administration of estradiol.

Group comparisons	Mean group difference	p-value
Group-I vs. Group-II	13.84 ng/ml	< 0.05
Group-I vs. Group-III	7.37 ng/ml	< 0.05
Group-II vs. Group-III	6.47 ng/ml	< 0.05

DISCUSSION

The present study evaluated the difference in stress responsiveness of HPA axis in control and Oophorectomized female Sprague Dawley rats. Serum corticosterone levels were measured under basal as well as stress induced conditions.

Stress results from exposure of an animal to hostile environment. A common working definition of physiologists is that "stress" consists of stimuli from the environment that tend to displace homeostasis and are perceived to be a threat or potential threat and animals initiate stress responses with activation of hypothalamo-pituitary adrenal axis and secretion of glucocorticoid hormones (cortisol and corticosterone).¹³

The results of the present study are consistent with that of Maklad *et al.* who studied the effect of forced swimming as a stress protocol on the markers of HPA axis reactivity.¹⁴ The rats were forced to swim for a period of 60 seconds in a plastic cylinder filled with water at a temperature of 21°C. The results indicated that exposure to swim stress was associated with a significant increase in serum corticosterone concentration as compared to the baseline values.

The stress induced increase in serum corticosterone levels as Oophorectomized female rats in this study, as compared to control rats, is comparable to the research work conducted by Hiramoto *et al.* They employed fasting as a stress procedure to study the changes in stress hormone levels following a period of 3 days stress.¹⁵ In contrast to the present study, they studied the effect of fasting stress in 8-week old mice and also studied the effect of stress on immunological parameters such as immunoglobulin A in the intestinal mucosa. The results showed a significant increase in the plasma levels of cortisol following 3 days fasting in Oophorectomized female rats as compared to the control group.

Two weeks of chronic restraint stress in this study resulted in increased levels of serum corticosterone in both the groups of Oophorectomized female Sprague Dawley rats. The stress induced increase in the study parameter was different in both the groups on account of estradiol administration to the group of Oophorectomized rats.

Oophorectomy in female rats mimics the normal physiological mechanism of menopause in the human

females and surgical removal of ovaries leads to physiological hypoestrogenism in female Sprague Dawley rats. During menopause, production of estrogen and progesterone gradually decreases.^{16,17}

Multiple mechanisms have explained augmentation of HPA responsiveness in estradiol treated Oophorectomized female rats. The important ones being the altered transcription of CRH mRNA at the level of paraventricular nucleus in the hypothalamus. The estrogen mediates its effects by binding to α and β -receptors, expressed in target tissues centrally and peripherally. Such effects may reflect the presence of estrogen response element in CRH gene promoter area and hence modulating the actions of estrogen on CRH gene expression.¹⁸

The levels of plasma corticosterone depend upon its rate of release from the adrenal cortex, the levels of transcortin or cortisol binding globulin (CBG) and its hepatic clearance and metabolism.

Since the levels of CBG in these female rats were not measured, therefore, the possible direct modulatory effect of estrogen replacement on plasma CBG levels and hence plasma steroid levels could not be ascertained. However, the estrogen treated female rats exhibited a clear cut rise in the free corticosterone levels after Oophorectomy.¹⁹

The possible cause of increase in serum corticosterone levels could partly be the result of enhanced adrenal synthesis of corticosterone under the effect of estradiol. The underlying mechanism could be the increased expression of melanocortin type-2 receptor in the stress exposed Oophorectomized rats.²⁰

Moreover, the enhanced secretion and release of corticosterone in females could not be explained by mere gender variations in the hepatic clearance. Female rats exhibited more rapid clearance of corticosterone that may result in a decreased rather than increased circulating levels of the hormone. Therefore, increased responsiveness of adrenal cortex in Oophorectomized rats could be due to mechanisms other than changes in metabolism and clearance.

Estrogen receptor alpha are also present on the adrenomedullary cells but it has not been known yet whether estrogens stimulated increase in corticosterone is due to its direct effect on adrenocortical cells or indirect effects on adrenomedullary cells.²¹

CONCLUSION

It is concluded that chronic restraint stress results increases the serum corticosterone levels in Oophorectomized Sprague Dawley rats and estradiol treatment increases the responsiveness of adrenal cortex of Oophorectomized female rats.

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