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Animal Research Section

Effect of Various Physical Stress Models on Serum Cortisol Level in Wistar Rats

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ABSTRACT

Background: Stress indicates the response or reaction of an organism to the environmental circumstances and their outcomes. Acute stress is well known to trigger several hormonal alterations in animals. An increase in glucocorticoid concentration can represent intensity of discomfort or distress experienced by an animal. The study was undertaken to evaluate the effects of various physical stress models on serum cortisol level in Wistar male rats.

Methodology: In this study six Wistar male rats weighing 150-200 gm were randomly selected. Animals were exposed to 'forced swim test' and 'restraint test'. Their serum cortisol level was measured by ELISA test using alpha prime ELISA system before and after the tests respectively.

Results: Results were analyzed by students paired t-test. Serum cortisol level was significantly higher after forced swim test as well as after restraint test. When both the physical activities were compared, serum cortisol level was increased more after restraint stress than after forced swim test however, the difference was not significant statistically.

Interpretation and Conclusion: The rise in serum cortisol level was observed in both the physical activity models . Rise in serum cortisol level was significantly higher after restraint test than exposing them to forced swim test. This indicates that restraining the rats produced more stress than making them forcefully swim.

Keywords: Forced swim test, Restraint test

INTRODUCTION

The term 'stress' used in behavioral research indicates 'a physical, chemical, or emotional factor to which an individual fails to make a satisfactory adaptation' [1]. It affects immune system, cardiovascular responses and many other systems. A multitude of hormones such as adrenocorticotrophic hormone (ACTH), glucocorticoids (GCs), and catecholamines (CA) are involved in the stress response [2].

Normally in animals, habitats are not constant and animals get adapted to predictable situations by physiological, morphological and behavioral modifications. The unpredictable components of life result in changes in the endocrine and metabolic status of an animal [3].

Understanding the effects of stress hormones give an idea about how stressors (any environmental perturbation that disrupts homeostasis) can affect the survival and reproductive success of living animals [4].

Acute stress is well-known to trigger marked increase in glucocorticoid concentration in blood. The hypothalamic–pituitary–adrenal (HPA) axis is the key hormonal system that has well-characterized circadian pattern. Under the influence of stress, this pattern is altered and it exerts adverse impact on health. Also degree of activation of the HPA axis is related to the intensity of stress experienced by animals [4]. Rats are very sensitive to stress, and a mild stressor results in a robust increase of glucocorticoid levels that approximates to the amplitude of diurnal rhythm [5].

Many studies have recently focused on stress as a marker of an animal's well being [6,7]. Since animals respond to a stressor by increasing their glucocorticoid (GCs) levels, there has been much interest in measuring these hormones [6].

A number of techniques have been used to measure GCs concentrations and these include sampling blood and feces. Blood GC concentrations have been used as an index of stress in a wide range of studies [7].

Thus this study was planned to detect the effect of different stress inducing, unpredictable physical activities on serum cortisol level in male rats.

MATERIALS AND METHODS

The project was approved by institutional animal ethical committee. Research Council's guidance for the care and use of laboratory animals was followed. For this study six wistar male rats were randomly selected. Their average weight was 177.3±20.37gm. Animals were subjected to forced swim test and restraint test with wash out period of 15 days. Experiments were conducted between 1 to 4 p.m on weekdays from July 2012 to September 2012.

Forced swim test: The plexiglass cylinder was filled with lukewarm water (25^{0}C) to a height of 15 cm. Rat was placed in water filled cylinder and allowed to step on moving wheel for survival. Rat was judged to be immobile wherever it remained floating in water in a slightly upright position, making no movements except trying to keep its head above water surface [8].

Restraint stress test: Animals were isolated once for 3 hours in a restraint transparent tube measuring 21cm x 7 cm [8].

Blood was collected from all the study group animals before and after each test.To characterize the cortisol response to an acute stressor, samples were taken at fixed timings.

Two ml of blood sample was collected by retro orbital puncture with all aseptic precautions in a plain vacutainer. It was allowed to clot for 30 minutes at room temperature. The Serum was separated by centrifugation at 2500 rpm for 5 minutes and subsequently stored

at -20°C. The serum samples were then subjected to estimation of serum cortisol. The hormone was estimated by automated chemiluminescence immunoassay system Alpha Prime LS, France by using Cortisol ELISA kit (DRG,USA). This immunoassay kit allows for in-vitro quantitative determination of endogenic cortisol concentration in serum. Principle of estimation of cortisol was competitive inhibition enzyme immunoassay technique. This assay has high sensitivity and specificity for estimation of cortisol levels in Wistar rats.

RESULTS

After exposure to forced swim test statistically significant ($p< 0.05^*$) rise in serum cortisol level was observed in Wister male rats [Table/Fig-1].

Also when same Wistar male rats were exposed to restraint stress a statistically significant rise in serum cortisol level was observed [Table/Fig-2].

Comparison of serum cortisol levels after forced swim test and after restraint stress was done in study group animals. It was observed that serum cortisol level was comparatively increased more after restraint stress [Table/Fig-3].

Parameter	Before Forced Swim Test (n=6)	After Forced Swim Test (n=6)
Serum cortisol (ng /ml) (Mean ±SD)	76.22±21.05	121.64±25.85*

[Table/Fig-1]: Effect of forced swim test on serum cortisol levels in male Wistar rats
p< 0.05*--Statistically significant

Parameter	Before Restraint Stress (n=6)	After Restraint Stress (n=6)
Serum cortisol (ng /ml) (Mean ±SD)	76.22±21.05	133.85±20.83**

[Table/Fig-2]: Effect of acute restraint stress on serum cortisol levels in male Wistar rats
p< 0.001**--Highly significant

Parameter	After Forced Swim Test (n=6)	After Restraint Stress (n=6)
Rise in serum cortisol (ng /ml) (Mean ±SD)	69.27±12.0	77.58±8.09

[Table/Fig-3]: Comparison of rise in serum cortisol level after forced swim test and after restraint stress in male Wistar rats
p> 0.05 –Statistically not significant

DISCUSSION

Present study was planned to evaluate effect of acute stress on serum cortisol level with different physical activity models inducing stress in Wistar rats. As shown in [Table/Fig-1] serum cortisol levels were significantly higher after forced swim test in male rats. Similar results were observed by Jiang Ya-Q et al., [9] and Preeti Kothiyal et al., [10] They observed that glucocorticoid levels were increased when rats were subjected to forced swim test till complete exhaustion. They concluded that stress in optimum quantum acted as stimulator to achieve the best, but when it exceeded, it caused imbalance in biochemical parameters as well as it lead to suppression in physical endurance.

In another study, 25 minutes of forced swim stress caused increase in glucocorticoid levels. Contarteze RVL et al., [11] observed that greater the intensity of activity, higher was the increase in the activity of the HPA axis during acute forced swim test.

[Table/Fig-2] shows significant rise in serum cortisol levels after acute restraint stress in male rats. Similar findings have been observed by Cho et al., [12] after applying the acute restraint stress for 90 min. Various studies have shown similar results. Garry Whitehead et al., [13] highlighted positive effects of glucocorticoids at both cellular and behavioral levels. However, they concluded that severity of the stressor was of central importance.

Study done by Pinnock and Herbert [14] reveled that animals exposed to single-stress (one period of 1-hour of acute restraint stress) showed increase in CRF levels. These findings were explained by estimation of corticotropin-releasing factor mRNA. They concluded that acute (single) restraint stress has differing effects 6 hours later on the expression of the mRNAs for CRF and arginine vasopressin in the paraventricular nucleus.

Statistically significant increased levels of serum cortisol after the tests suggest that a cascade of neurohumoral events occur chiefly at the level of the hypothalamic-pituitary-adrenocortical (HPA) axis. The HPA axis is the pivot for the animal's ability for adaptation and coping with stress. Increase in the cortisol level causes termination of the stress reaction leading to homeostasis. Depending on intensity and duration of physical activity cortisol level changes as it plays a role in tissue recovery and repair [15].

Reviews of early studies in humans, rodents and nonhuman primates concluded that situations characterized by novelty, unpredictability or low perceived control were most likely to activate the HPA axis [16].

As shown in [Table/Fig-3] comparison of rise in serum cortisol levels between forced swim test and restraint stress is not statistically significant in male rats. These observations have indicated that both the tests exhibited comparable amount of stress in male rats.

Thus cortisol, the major stress hormone, serves as a key controller for neurohumoral responses which underlie behavioral adaptations. Stressors–whether they are physical or psychological activate afferent neural pathways within the central nervous system which project to diencephalic centres where they initiate a response [17]. This response may be behavioral, autonomic and/or endocrine.

LIMITATIONS

For estimation of the Serum cortisol levels samples were taken in early evening at 5 to 6 pm. We could not take the samples in late evening after 7 pm when rats peak activity begins. In this study female rats were not included.

Future scope: Considering the effect of restraint stress on Serum cortisol levels in rats, cortisol levels can be mesured in sedentary workers having stress and they should be made aware about the side effects of increased cortisol levels.

CONCLUSION

Our study showed that any stress, associated with or without physical activity, increases cortisol level. Though not statistically significant, cortisol level was found to be raised more when physical activity was restricted rather than forced physical activity.

REFERENCES

[1] Romero ML, Butler Luke K. Endocrinology of Stress. *International Journal of Comparative Psychology*. 2007; 20: 89-95. www.springer.com/978-1-58829-432-6.

[2] Matteri RL, Carroll JA, Dyer CJ. Neuroendocrine responses to stress. In: Moberg GP, Mench JA, editors. The Biology of animal stress. CABI Publishing. 2000. p. 43–76.

[3] E. Möst, R. Palme Hormones as indicators of stress. *Domestic Animal Endocrinology*. 2002;23: 67–74.

[4] Gambarana C. Experimental protocols for the study of stress in animals and humans. Nutrients, stress and medical disorders. (Eds) Yehuda S and Mostofsky D Human press Inc Totowa. 2005; 21-30.

[5] Elizabeth A. Younga,b, James Abelsona, Stafford L. Lightman. Cortisol pulsatility and its role in stress regulation and health. *Frontiers in Neuroendocrinology.* 2004;25 : 69–76.

[6] Terlouw EM, Schouten WGP, Ladewig J. Physiology. In: Appleby MC, Hughes BO, editors. Animal welfare. Cambridge: CAB International, University Press, 1997. 143–58.

[7] Michael J. Sheriff AB, Charles J. Krebs b, Rudy Boonstra. Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? *General and Comparative Endocrinology*. 2010;166: 614–19.

[8] Jaggi AS, Bhatia N, Kumar N, Singh N, Anand P, Dhawan R. A review on animal models for screening potential anti-stress agents. *Neurological Sciencess*. 2011; 32(6):993-1005.
[9] Jiang YQ, Kawashima H, Iwasaki Y, Uchida K, Sugimoto K, Itoi K. Differential effects of forced swim-stress on the corticotropin-releasing hormone and vasopressin gene transcription in the parvocellular division of the paraventricular nucleus of rat hypothalamus. *Neuroscience Letters*. 2004; 358: 201–04.
[10] Kothiyal P, Ratan P. Antistress Effect of Fagopyrum esculentum in rats subjected to forced Swimming endurance test. *Pharmacology online*. 2011; 3:290-96.
[11] Contarteze RVL, Manchado FB, Gobatto CA, Mello MAR. Biomarkers of stress in rats exercised in swimming at intensities equal and superior to the maximal estable lactate phase. *Revista Brasileira de Medicina do Esporte*. 2007; 13:150-54.
[12] Cho YJ, Kim JH, Yim HE, Lee DM, Im SK, Lee KJ. Role of corticotrophin-releasing factor in the stress-induced dilation of esophageal intercellular spaces. *Journal of Korean medical science*. 2011; 26: 279-83.
[13] Garry Whitehead, Jihoon Jo, Ellen L. Hogg, Thomas Piers, Dong-Hyun Kim, Gillian Seaton, et al. Acute stress causes rapid synaptic insertion of Ca2+-permeable AMPA receptors to facilitate long-term potentiation in the hippocampus.*Brain*. 2013; 136(12): 3753–65.
[14] Pinnock SB, Herbert J. Corticosterone differentially modulates expression of corticotropin releasing factor and arginine vasopressin mRNA in the hypothalamic paraventricular nucleus following either acute or repeated restraint stress. *European Journal of Neuroscience*. 2001; 13(3): 576–84.
[15] Selye H, Fortier C. Adaptive Reaction to Stress. *Journal of Biobehavioral Medicine*. 1950; 12(3):149-57.
[16] Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE. The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition*. 2007; 65:209–37.
[17] Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*. 2009; 10:397-409.

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