EFFECT OF ALPHA TOCOPHEROL ON CHRONIC STRESS INDUCED DERANGEMENTS IN NEUROPEPTIDE Y ON SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To determine the chronic stress induced decline in plasma Neuropeptide Y (NPY) level and protective effects of Alpha Tocopherol (AT) on maintenance of Neuropeptide Y level.
Study Design: Quasi-experimental study.
Place and Duration of Study: The study settings were Al-Nafees Medical College and animal center of National Institute of Health Islamabad (NIH). Duration of research was one year from May 2015 to May 2016.
Methodology: After taking approval of institutional review board 45 male Sprague Dawley rats with inclusion criteria were chosen and allocated to three groups comprised of n=15. Group I was control, group II restraint stress and group III (restraint stress with supplementation of alpha-tocopherol). After 28 days of restraining stress induction plasma Neuropeptide Y level was determined by sandwich Enzyme Linked Immunosorbent Assay (ELISA).
Results: There was a statistically significant decline (p<0.05) in plasma Neuropeptide Y levels in restraint group as compared to control group and with alpha-tocopherol supplementation group.
Conclusion: Chronic restraint stress induction led to decline in Neuropeptide Y levels and alpha-tocopherol prevented fall in Neuropeptide Y levels.

Keywords: Alpha-Tocopherol, Neuropeptide Y, Sprague dawley rats.

INTRODUCTION

Stress in either form physical or psychological activates certain body systems which enables body to cope with given circumstances but when this coping reaction is inadequate in intensity or dysregulated, an imbalance arises between individual's capabilities to cope with stress and burdens and strains that are imposed by stress[1].

Pertaining to duration, stress can be acute, episodic and chronic. Stress exposure for more than week stated as chronic stress and has been described in animal models. Repeated daily induction of restraining stress for 21 days and 28 days has been well described in rats' studies[2,3].

Body responds to acute and chronic stress differently[4]. In terms of effects it can be "Eustress" (good stress) which is beneficial and required to boost and motivate individuals to accomplish a task. Contrariwise "Distress" is characterized by negative attitude, places burden on the body and ends up with its dysfunction such as hypertension, migraine headaches, muscular pains and several other ailments. Under normal physiological conditions "Eustress" is essential for better performance without development of dysfunction[5].

Research studies have pinpointed mainly three tripartite systems which are activated by stress. These systems are sympathetic nervous system with adrenal medulla, hypothalamus tied with spinal cord and adrenal gland along with the system of hypothalamus linked with pituitary gland and adrenals. The intricate stress reaction is intervened by the release of cortisol and NPY. The functional interplay of NPY with cortisol and other stress hormones stabilizes many functions of the body to fight in danger, to adapt and be more resilient in stressful conditions[6].

It has been found that body metabolism is adversely affected by intense stress, culminating to drastic acceleration of metabolic process which

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includes toxic derivatives of oxygen metabolism. Homeostasis of reduction and oxidation reactions remain not in normal limits and initiate chain reactions of lipid peroxidation of cell membrane and membranes of cellular organelles[7].

Repeated and prolonged activation of hypothalamus in chronic stress leads to release of norepinephrine (NE), corticotropin releasing factor (CRF), glutamate, arginine vasopressin and GABA along with NPY[6]. The NPY release is a measure of sympathetic activity because it co-localizes and co-stores in catecholamine secreting neurons. It is proposed that there exists reciprocal functional interplay of NPY with other pro stress neurotransmitters (GABA, glutamate, arginine vasopressin, corticotropin releasing factor, norepinephrine). Thus, it opposes the effects exhibited by stress hormones. NPY hampers adenylyl cyclase activity, reduces cAMP concentration and deactivates basolateral amygadalar pyramidal cells while CRF depolarizes them. Thus NPY released in brain regions are activated in the regulation of anxiety, and stress[8].

It has been determined that NPY-ergic system modulates stress effects differently in individuals because of contribution of genetic and environmental factors in regulation of stress response. However, dysregulation ends up dysfunction and disease process[9].

Alpha tocopherol an antioxidant which is fat soluble and has easy access to cell membrane, stops production of lipid radicals. Alpha tocopherol is a part of cell membrane interior and readily removes reactive oxygen species (ROS) when comes in contact with ROS. Although a large array of data points out the modulation of NPY-ergic system in stress response but understanding of its dysregulation for further development still needs elucidation[10]. In the current study we determined the effect of chronic stress with and without administration of AT on NPY release.

METHODOLOGY

The study design was quasi-experimental and place of the study Al-Nafees Medical College and Hospital but animal house of National Institue of Health (NIH) Islamabad was selected for animal housing and experimentation. It started from May 2015 and ended May 2016. Permission from institutional review board was taken prior to the start of experimentation. Sampling technique was non probability convenience sampling. Sample size was calculated on the basis of observation that decreased NPY levels in chronic restraint stress at baseline and after induction of stress as effect size (difference of means and standard deviations). Effect size was used as prerequisite for sample size calculation. With 95% confidence interval, power of 80, difference of means between the baseline and after chronic stress and standard deviations, sample size was calculated by open epi calculator[11-13].

Demographic features included age and mean body weight of each group. Forty-five male SD rats with 275 ± 25 gm weight and age of 90 to 120 days, purchased from NIH Islamabad, were included in the study. All rats remain healthy throughout the study period.

Acclimatization: A period of one week was required for all rats to acclimatize with environmental factors which included well maintained and recommended humidity and room temperature of 22 ± 2°C. Exposure to day light and night period was kept at 12:12-h. Animal food and tap water were given as per recommendations of animal center. Grouping and coding of all rats were done after acclimatization period. After allocation to three arms of the study the groups were: group I (control, n-15), group II (restraint, n-15), group III (restraint stress + alpha tocopherol with dose of 50mg/kg by oral route daily for 28 days, n-15).

Experimentation: Immobilization and food deprivation were the components of restraining stress. Wire mesh restrainers with dimension of 18x8x8 cm[12] were used to induce stress. After one week of adaptation rats were subjected to restraint stress individually for uninterrupted 28 days with time period of 6 hours each day[13].

Restraint stress was applied to group II and III (experimental groups).

Blood Sampling: At terminal stage of the study, ether as an anesthetic agent was used to anesthetize each rat. A 19 gauge needle was inserted into the heart and blood sample was taken. Samples were transferred to separate vacutainers containing EDTA and centrifuged for 15 minutes at 3000 rmp at 4°C and plasma was kept for storage at -20°C and for quantification of NPY by ELISA with Cat. # EZRMNPY-27K.

Statistical analyses were done by SPSS-20 and comparison among groups was done by one-way ANOVA for age and weight distribution among three groups. Plasma concentrations NPY in three groups were compared by one-way ANOVA and Post Hoc Tukey's test.

RESULTS

Induction of chronic restraint stress led to decline in plasma NPY in group II. Results obtained from group I and III of plasma NPY levels were comparable. Mean value of plasma NPY level of group I denoted as control was 0.619 ± 0.05 ng/ml. Rats of the restraint stress group had mean plasma NPY level of 0.357 ± 0.07 ng/ml, whereas experimental group with supplementation hadmean plasma NPY level of 0.570 ± 0.05 ng/ml.

Statistically insignificant results were found when age and weight distribution were analyzed among three groups, $p>0.05$ (table-I). For comparison of NPY concentrations among three groups One-way ANOVA test was applied which revealed significant difference upon comparison, $p<0.05$ (table-II). So, ANOVA Post Hoc Tukey's test was applied. Statistically significant difference was obtained when mean value of NPY concentration of group II was compared with that of control group, $p<0.05$ (table-III). When group II was compared with group III, significant result was obtained $p<0.05$ (table-IV), whereas upon comparison of control with group III revealed insignificant result, $p>0.05$ (table-III).

Table-I: Comparison of demographic parameters among three groups by one-way ANOVA.

	Groups I n=15	Groups II n=15	Groups III n=15	*p*-value
Ages of rats in days (Mean ± SD)	101 ± 10	108 ± 8	103 ± 8	0.156
Weights of rats (Mean ± SD) before experimentation in grams	282 ± 16	283 ± 14	285 ± 15	0.868

Table-II: Comparisons of plasma Neuropeptide Y concentrations of group I with groups II, III (ANOVA).

Groups	Groups I n=15	Groups II n=15	Groups III n=15	*p*-value
NPY levels ng/ml. (Mean ± SD)	0.619 ± 0.05	0.357 ± 0.07	0.570 ± 0.05	0.001***

Table-III: Comparisons of plasma Neuropeptide Y concentrations of group I with groups II, III (ANOVA Post Hoc Tukey's test).

Parameters of group-I n=15	Mean ± SD	Comparison of Group-I with Other Groups-II, III	
Neuropeptide Y	0.619 ± 0.05	0.001***	0.212

Table-IV: Comparisons of plasma Neuropeptide Y concentrations of group II with group III (ANOVA Post Hoc Tukey's test).

Parameters of group II n=15	Mean ± SD	Comparison of Group II with Group III, III
Neuropeptide Y	0.357 ± 0.07	0.001***

DISCUSSION

The present study showed results of plasma NPY levels in control group which are in agreement with Grise *et al*[13] who showed similar trend of plasma NPY levels in male SD control

group rats using ELISA kit. The results are also in consistent with Hassan *et al*[14] who determined NPY levels by ELIZA in control group of mice with chronic stress paradigm. Similarity of results may be due to the similar patterns immunoassay used, however, result differs from Lijun *et al*[15] who showed baseline NPY levels in control group by ELISA method in male wistar rats. The difference may be due to different species of rats.

In our experimental model, rats of group II, exposed to restraint stress showed low plasma NPY levels. Daubert *et al*[16] have reported 2.5 times rise in NPY concentrations upon induction of acute stress but there was noticeable diminution in NPY rise when rats were subjected to chronic stress. In this study stressed group with implanted corticosterone releasing pellets in

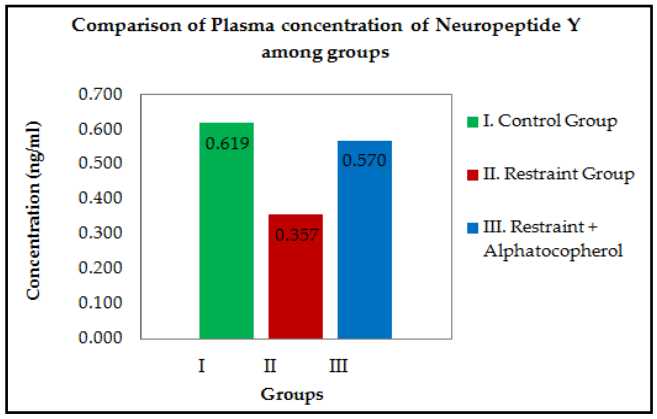

Figure: Comparisons of plasma Neuropeptide Y concentrations among all three groups.

hind brain was contrasted with stressed group had implantation of sham pellets. This decreased plasma NPY levels could be due to prolonged oxidative stress. Prolonged use of opioids shows greater propensity to develop stress linked adverse effects by slow down the formation of mRNA levels of NPY and its dysregulation. On the contrary amphetamines users exhibit high levels of NPY mRNA. Tissue hormonal mRNA concentrations has been found to be linked with plasma hormonal levels[17]. Yohimbine is an alpha-2 blocker, it has been found in a research project that it hampers NPY rise in plasma in patients with posttraumatic stress contradistinguished to healthy participants[18].

Our study is contradictory to Rasmusson *et al*[19] who showed non-significant rise in plasma NPY levels after 12 days of chronic immobilization stress induced on wistar rats neither at baseline nor after acute foot shock stimulation because of lesser duration of restraint stress exposure, however, NPY levels were increased in with chronic stress alone and in combination of stress and high sodium chloride diet.

Our study showed statistically insignificant difference in NPY levels in group-III that subjected to restraint stress which were given AT compared to control group. These results are comparable to findings by Hounsom *et al*[20] who showed NPY depletion in rats those had dietary deficiency of alpha tocopherol as compare to animals of control group, showing a link between oxidative stress and NPY depletion. The beneficial effects of AT supplementation revealed improvement in magnitude of oxidative stress in rodent model of male wistar rats through antioxidant action of AT[21].

AT is present in inner structure cell membrane and on surface of lipoproteins, removes harmful and damaging radicals produced by oxygen metabolism in many diseases along with stress related disorders[22]. AT protects polyunsaturated fatty acids of cell membrane and considered chain breaking antioxidant radical when comes in contact with lipid peroxide radical of damaged membrane resulting membrane stability and improves cell functions including secretory activity[23]. So, of relating to effects of AT on NPY, research revealed that AT reduces fall in NPY concentrations in chronic stress[24].

AT supplementation had better treatment outcomes in stress ulcers of stomach when paralleled with ulcer healing without supplementation. This could result due to combined effects of functional break exerted by NPY on stress hormones and by sparing action of AT on NPY release[25].

AT has propensity to interact with cellular antioxidants, antioxidant enzymes to inhibit

oxidative stress. The increase in oxidative stress is linked with several disease processes such as stress disorders, cancers, and diabetes mellitus. The pharmacological, and medicinal characteristics of AT render it a potential therapeutic agent in management of stress linked and other diseases.

CONCLUSION

Chronic restraint stress induction leads to decrease in NPY levels and AT prevents a fall in NPY levels.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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