EFFECT OF CHRONIC STRESS ON NEUROPEPTIDE Y AND OXIDATIVE STRESS.

Saadia Zainab1, Umar Ali Khan2, Tahir Ahmad Munir3, Anjum Ilahi4, Adnan Saleem Khan5, Ayesha Javed6

ABSTRACT: In chronic stress, release of catecholamines, adrenocorticoids and pituitary hormones result impaired release of neuromodulator - neuropeptide Y. The deregulated neuropeptide Y results imbalanced redox homeostasis reduced endogenous superoxide dismutase and raised malondialdehyde. Objectives: To find the effect of chronic stress on plasma neuropeptide Y, superoxide dismutase, and malondialdehyde levels. Study Design: Quasi-experimental. Setting: Al-Nafees Medical College & Hospital in collaboration with National Institute of Health, Islamabad. Period: January 2016 to December 2016. Material & Methods: After approval from institutional review board, thirty healthy male Sprague Dawley rats were included in the study and were divided equally into group I (control) and group II (restraint stress). The animals were housed in stainless steel cages, at humidity (40-60%), temperature (22 ± 2°C) and a 12-h light-dark cycle with lights on at 0700 am. After adaptation, group II was exposed to restraint stress of 6 hours daily for 28 days. The blood sampling for plasma neuropeptide Y, serum superoxide dismutase and malondialdehyde levels were taken. Results: There was significant decline in neuropeptide Y plasma and superoxide dismutase serum levels while an increase in malondialdehyde levels serum levels was noticed in restraint stress group. Conclusions: Chronic stress induces decrease in plasma neuropeptide with subsequent increase in serum malondialdehyde and decrease in superoxide dismutase levels. Key words: Malondialdehyde, Neuropeptide Y, Restraint Stress, Superoxide Dismutase.

INTRODUCTION

Stress, an imbalance between demands and resources, is caused either by physical or psychological demands of organism that exceed its capabilities and resources to fulfill the demands. A physical damage to the body results physical stress while psychological stress includes awareness of psychological harm.1

The stress can be good as seen in motivational situation and toxic or distress which is almost always negative and harmful, is disease related and may end up with depression, anxiety, mood swings, muscular pains and cardiovascular dysfunction.2

The physiological response of neuropeptide Y (NPY) called general adaptation syndrome is mediated by G-protein coupled receptors - Y1, Y2, Y4, and Y5, which on activation mobilize enzyme linked calcium or have myriad actions on ion channels. The NPY receptors, located in cortex, amygdala, hypothalamus, and locus coeruleus, modulate functions of gamma aminobutyric acid (GABA), glutamate, corticotrophin-releasing factor (CRF), and norepinephrine (NE) secreting neurons.3

Stress induced adrenal gland stimulation raises blood cortisol level – glucocorticoids that dysregulate redox homeostasis, alter metabolism and antioxidant like superoxide dismutase (SOD), glutathione and others.4 The malondialdehyde (MDA), a biomarker of lipid peroxidation, increases in oxidative stress.5 The reactive oxygen species (ROS), a leading cause...
of oxidative stress, is formed either directly from mitochondrial membrane or as a metabolic byproduct of mutated SOD through glutathione pathway.³

A change of tissue NPY content with increase in oxidative stress parameters have been well documented⁶-⁷, but plasma NPY derangements with oxidative stress parameters still need elucidation. The objective of this study was to find the effect of chronic stress on plasma NPY and oxidative stress (SOD, MDA) levels in Sprague Dawley rats.

MATERIAL & METHODS
This quasi-experimental study was conducted at Al-Nafees Medical College and Hospital, Islamabad, in collaboration with National Institute of Health Islamabad from January 2016 to December 2016 after approval from institutional review board. Thirty (N= 30) Sprague Dawley male rats were included in the study, and were equally divided into two group I (control) and group II (restraint stress). Based on the previous data on chronic stressed induced decline in NPY levels, sample size was calculated with power 80, 95% confidence interval and difference of means between stressed and non-stressed groups by open epi calculator.⁸

The rats of group II (restraint stress) were exposed to restraint stress by wire-mesh restrainer 6 hours daily for continuous 28 days. At the end of restraining, each rat was anesthetized in a closed chamber with ether soaked cotton and about 4 ml blood was drawn by cardiac puncture. Blood was transferred to separate vacutainers, containing anti-coagulant EDTA for plasma and thrombin based clot activator for serum. The samples were centrifuged at 3000 rpm for 15 minutes. Plasma was stored in eppendrofs at -20°C for NPY analysis by ELISA; while serum samples were stored at -80°C for SOD and MDA colorimetric analysis.

RESULTS
Results were analyzed by SPPSS 20. A total of 30 Sprague Dawley healthy male rats were included with a mean age of 104±9 days and a mean weight of 283 ± 14 gm.

Mean ±SD of plasma NPY, serum SOD and MDA levels are shown in the table. In group I, the mean plasma NPY levels was 0.619± 0.05 ng/ml, the mean serum superoxide dismutase levels was 0.020± 0.010 U/ml while the mean serum malondialdehyde value was 1.36± 0.2 µM. Regarding group II, the mean NPY level was 0.357± 0.08 ng/ml, serum superoxide dismutase showed a level of 0.009±0.002 U/ml while serum mean malondialdehyde levels were 7.47± 0.2 µM.

Multivariate test MANOVA (multiple analyses of variance) was applied to find the significant difference. A statistically significant difference (p<0.05) is seen when NPY, SOD and MDA levels were compared between group I and II.

DISCUSSION
The results obtained by current study have been in agreement with a number of the studies which showed increased baseline plasma NPY levels in control group compared to stress induced group. Increased NPY plasma levels were determined in control group in contrast to restraint stress group in a study of rodent model and similar findings were obtained in starved rats in comparison with non-starved rats as starvation and immobilization are components of restraint stress.⁹-¹⁰

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (n- 15) Mean ± SD</th>
<th>Group II (n- 15) Mean ± SD</th>
<th>95% C I</th>
<th>P-Value</th>
</tr>
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<tbody>
<tr>
<td>Neuropeptide Y (ng/ml)</td>
<td>0.619± 0.05</td>
<td>0.339± 0.08</td>
<td>0.225 – 0.335</td>
<td>0.000</td>
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<tr>
<td>Superoxide dismutase (U/ml)</td>
<td>0.020± 0.010</td>
<td>0.009± 0.002</td>
<td>0.0058 – 0.0176</td>
<td>0.001</td>
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<tr>
<td>Malondialdehyde (µM)</td>
<td>1.362± 0.28</td>
<td>7.471± 0.26</td>
<td>-6.314 – 05.903</td>
<td>0.000</td>
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Table. Comparison between group I and group II of NPY, Serum SOD and MDA levels
Baranowska et al, and Christiansen et al showed increased baseline NPY levels in plasma in non-starved and starved rats. The results of present study are not in line with Eshkevari et al who declared an elevated plasma NPY levels in rats exposed to 14 days cold stress compared to levels at day 0. The contradictory results have been obtained probably due to lesser duration stress induced by different stressor. Moreover, the results are not in agreement with the study conducted by Xu et al who showed an increased NPY levels in stress induced rats compared to baseline levels; probable reason may be use of different stressors in sequence for different time duration.

Our results are similar with a number of studies which showed decreased SOD and increased MDA levels in rats exposed to restraint stress. Induction of chronic restraining stress of 21 days led to increase in MDA and decrease in SOD concentrations. Similar trend of decrease in SOD levels has been found when rats were subjected to chronic restraining stress of 21 days and increase in MDA concentrations were also estimated.

In chronic restraint stress rats with low serum NPY levels, showed raised behavioral sensitivity to normal anxiety, increased susceptibility to non-epileptic seizures and fear suppression. The study depicts that low serum NPY levels have been associated with decreased response of NPY to induce anxiolytic and anti-stress effects in chronic stress. The present study has shown similarity to the study conducted by Rasmusson et al in which low plasma NPY associated chronic stress of combat related post-traumatic stress disorders in male was determined. Reactivity of adrenergic system increases with low plasma NPY levels was depicted by stimulation of yohimbine to increase the symptoms of post-traumatic stress. Analogy of the phenomenon has been shown in a study conducted on medical students with academic chronic stress. In the study statistically significant decline in NPY was determined from baseline to terminal stage along with worsening of profile of mood states.

Plasma NPY levels from patient’s previous records were taken and comparison was done between healthy controls and suicide subjects. The study has provided contradiction to the results of present study probably due to lack of data about the exact duration of stress associated with suicide. Moreover, a change in NPY-Y1 receptors density was found on hippocampi depicting NPY system modulation in stress response. In animal model, a decreased NPY expression in selected brain regions is associated with disrupted behavior and anxiety. NPY mediated serum oxidative stress parameter showed similar trend in ischemic cardiomyocytes.

Chronic restraining stress leads to impaired cognitive function, increased neuronal plasticity or structural changes that lead to remodeling of NPYergic system to adapt to the stress. The neuronal plasticity contributes in stress related depression, anxiety and posttraumatic stress disorders.

The NPY directly decreases oxidative stress by stabilizing mitochondrial membrane, prevents loss of proteolysis, decrease stem cells exhaustion and impaired nutrient sensing. NPY indirectly decreases oxidative stress by controlling undesired effects of cortisol, catecholamine and others.

CONCLUSIONS
Chronic stress induces decrease in plasma NPY with subsequent increase in oxidative stress parameters reflected by increase in serum MDA and decrease in serum SOD levels.

REFERENCES


**AUTHORSHIP AND CONTRIBUTION DECLARATION**

<table>
<thead>
<tr>
<th>Sr. #</th>
<th>Author(s) Full Name</th>
<th>Contribution to the paper</th>
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<tbody>
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<td>Saadia Zainab</td>
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