The Professional Medical Journal
www.theprofesional.com

ORIGINAL PROF-0-3506

DOI: 10.29309/TPMJ/2020.27.05.3506

EFFECT OF CHRONIC STRESS ON NEUROPEPTIDE Y AND OXIDATIVE STRESS.

Saadia Zainab[1], Umar Ali Khan[2], Tahir Ahmad Munir[3], Anjum Ilahi[4], Adnan Saleem Khan[5], Ayesha Javed[6]

1. MBBS, M.Phil
Assistant Professor Physiology
Al-Nafees Medical College
Islamabad.
2. MBBS, M.Phil, FCPS, Ph.D
Professor Physiology
Al-Nafees Medical College
Islamabad.
3. MBBS, FCPS (Physiology),
Professor Physiology
Mohi-Ud-Din Islamic Medical
College Mirpur AJK.
4. MBBS, MRCP (Medicine),
FRCP (Medicine),
Dip Card, MRCP (Geriatric)
Associate Professor Medicine
Al-Nafees Medical College
Islamabad.
5. MBBS, Dip Card, MPH
Registrar and Postgraduate
Cardiology
Al-Nafees Medical College
Islamabad.
6. MBBS,
M.Phil
Assistant Professor Community
Medicine
Al-Nafees Medical College
Islamabad.

Correspondence Address:
Dr. Saadia Zainab
Department of Physiology
Al-Nafees Medical College Islamabad.
drsaadiakmu7@gmail.com

Article received on:
02/04/2019
Accepted for publication:
28/08/2019

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.

Article Citation: Zainab S, Khan UA, Munir TA, Ilahi A, Khan AS, Javed A. Effect of chronic stress on neuropeptide y and oxidative stress. Professional Med J 2020; 27(5):902-906. **DOI:** 10.29309/TPMJ/2020.27.05.3506

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 documented[6-7], 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]

Variables	Group I (n- 15) Mean ± SD	Group II (n- 15) Mean ± SD	95% C I	P-Value
Neuropeptide Y (ng/ml)	0.619± 0.05	0.339± 0.08	0.225 – 0.335	0.000
Superoxide dismutase (U/ml)	0.020± 0.010	0.009± 0.002	0.0058 – 0.0176	0.001
Malondialdehyde (μM)	1.362± 0.28	7.471± 0.26	-6.314 – 05.903	0.000

Table. Comparison between group I and group II of NPY, Serum SOD and MDA levels

Baranowska et al[11], and Christiansen et al[12] 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[13] 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[14] 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.[15] 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.[16]

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.[17] 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.[18] 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.[19]

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.[20] Moreover, a change in NPY-Y1 receptors density was found on hippocampi depicting NPY system modulation in stress response.[21] 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.[6,22]

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.[23]

The NPY directly decreases oxidative stress by stabilizing mitochondrial membrane, prevents loss of proteolysis, decrease stem cells exhaustion and impaired nutrient sensing.[24] NPY indirectly decreases oxidative stress by controlling undesired effects of cortisol, catecholamine and others.[25]

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

1. Schönfeld P, Preusser F, Margraf J. **Costs and benefits of self-efficacy: Differences of the stress response and clinical implications.** Neurosci Biobehav Rev. 2017; 75:40-52.

2. Shahsavarani AM, Abadi EA, Kalkhoran MH. **Stress: Facts and theories through literature review.** Int J Med Rev. 2015; 2(2): 230-41.

3. Schubert M, Stichel J, Du Y, Tough IR, Sliwoski G, Meiler J, et al. **Identification and characterization of the first selective Y4 receptor positive allosteric modulator.** J Med Chem 2017; 60(17):7605-12.

4. Trivedi MS, Holger DA, Bui AT, Craddock TJ, Tartar JL. **Short-term sleep deprivation leads to decreased systemic redox metabolites and altered epigenetic status.** PloS one. 2017; 24; 12(7):https://doi.org/10.1371/journal.

5. Spiers JG, Chen HJ, Sernia C, Lavidis NA. **Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress.** Front Neurosci. 2015;8;456e

6. Chen A, Li W, Chen X, Shen Y, Dai W, Dong Q, et al. **Trimetazidine attenuates pressure overload-induced early cardiac energy dysfunction via regulation of neuropeptide Y system in a rat model of abdominal aortic constriction.** BMC Cardiovas Disord. 2016; 16(1):225.

7. Mishra S, Mishra B. **Study of lipid peroxidation, nitric oxide end product, and trace element status in type 2 diabetes mellitus with and without complications.** Int J Appl Basic Med Res. 2017; 7(2):88-93.

8. Daubert DL, Looney BM, Clifton RR, Cho JN, Scheuer DA. **Elevated corticosterone in the dorsal hindbrain increases plasma norepinephrine and neuropeptide Y, and recruits a vasopressin response to stress.** Am J Physiol Regul Integr Comp Physiol. 2014; 307(2):R212-24.

9. Grisé KN, Olver TD, McDonald MW, Dey A, Jiang M, Lacefield JC, Shoemaker JK, Noble EG, Melling CW. **High intensity aerobic exercise training improves deficits of cardiovascular autonomic function in a rat model of type 1 diabetes mellitus with moderate hyperglycemia.** Journal of diabetes research. 2016;2016.

10. Eskandarzade N, Saeb M, Nazifi S, Saeb S, Kazemipour N, Ansari-Lari M. **The effect of long term starvation on galanin, leptin, thyroid hormones, insulin, prolactin, growth hormone, ghrelin and factors involved in energy metabolism in adult goats.** J Fac Vet Med Istanbul Univ. 2015; 41:143-50.

11. Baranowska B, Chmielowska M, Wolinska E, Roguski K, Wasilewska E. **The relationship between neuropeptides and hormones in starvation.** Neuro Endocrinol Lett. 2001; 22(5):349-55.

12. Christiansen SH, Olesen MV, Wörtwein G, Woldbye DP. **Fluoxetine reverts chronic restraint stress-induced depression-like behaviour and increases neuropeptide Y and galanin expression in mice.** Behav. Brain Res. 2010; 216 (2011)585–91.

13. Eshkevari L, Egan R, Phillips D, Tilan J, Carney E, Azzam N, et al. **Acupuncture at ST36 prevents chronic stress-induced increases in neuropeptide Y in rat.** Exp Biol Med 2012; 237(1):18-23.

14. Xu LZ, Liu LJ, Yuan M, Li SX, Yue XD, Lai JL, et al. **Short photoperiod condition increases susceptibility to stress in adolescent male rats.** Behavioral Brain Research. 2016; 300:38-44.

15. Abdulrauf RA, Dawud FA, Emmanuel NS, Muhammad HD, Dange AS, David BA, et al. **Lipid peroxidation and some antioxidant enzymes evaluation in apple cider vinegar (ACV) treated male and female wistar rats exposed to chronic restraint stress.** Advances in Enzyme Research. 2018; 6(3):21-8.

16. Samarghandian S, Azimi-Nezhad M, Borji A, Samini M, Farkhondeh T. **Protective effects of carnosol against oxidative stress induced brain damage by chronic stress in rats.** BMC Complement Altern Med. 2017; 17(1):249.

17. Winterdahl M, Miani A, Vercoe MJ, Ciovica A, Uber-Zak L, Rask CU, et al. **Vulnerability to psychogenic non-epileptic seizures is linked to low neuropeptide Y levels.** Stress 2017; 20(6):589-97.

18. Rasmusson AM, Hauger RL, Morgan III CA, Bremner JD, Charney DS, Southwick SM. **Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat-related PTSD.** Biol Psychiatry. 2000; 47(6):526-39.

19. Guidi L, Tricerri A, Vangeli M, Frasca D, Errani AR, Di Giovanni A, Antico L, Menini E, Sciamanna V, Magnavita N, Doria G. **Neuropeptide Y plasma levels and immunological changes during academic stress.** Neuropsychobiology. 1999; 40(4):188-95.

20. Lu J, Li S, Li H, Mou T, Zhou L, Huang B, Huang M, Xu Y. **Changes in plasma NPY, IL-1β and hypocretin in people who died by suicide.** Neuropsychiatr dis and treat. 2019; 15:2893.

21. Thorsell A, Michalkiewicz M, Dumont Y, Quirion R, Caberlotto L, Rimondini R, et al. **Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression.** Proc Natl Acad Sci 2000; 97(23):12852-7.

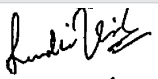
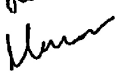
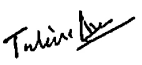
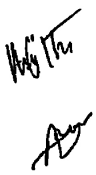
22. Tian X, Sun L, Gou L, Ling X, Feng Y, Wang L, et al. **Protective effect of l-theanine on chronic restraint stress-induced cognitive impairments in mice.** Behav Brain Res. 2013; 1503:24-32.

23. Tamashiro KL, Sakai RR, Shively CA, Karatsoreos IN, Reagan LP. **Chronic stress, metabolism, and metabolic syndrome.** Stress. 2011; 14(5):468-74.

24. Botelho M, Cavadas C. **Neuropeptide Y: An anti-aging player?.** Trends in neurosciences. 2015; 38(11):701-711.

25. Sabban EL, Alaluf LG, Serova LI. **Potential of neuropeptide Y for preventing or treating post-traumatic stress disorder.** Neuropeptides. 2016; 56:19-24.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Saadia Zainab	Principal investigator of the project, Project conduction, manuscript writing.	
2	Umar Ali Khan	Conception, study design and proof reading.	
3	Tahir Ahmad Munir	Substantial contributions to conception and designing the work, conduction of workshop and drafting the article.	
4	Anjum Ilahi	Revising it critically for important intellectual input and final approval fo the version to be publisehd.	
5	Adnan Saleem Khan	Substantial contributed in data analysis and interpretation.	
6	Ayesha Javed	Substantial contributed in data analysis and interpretation.