Original Article

Evaluation of Protective Role of Benzodiazepine in Noise Stress Induced Activation of Hypothalamo-Pituitary Adrenal Axis in Albino Rats

1. Syed Meesam Iftekhar.H.Rizvi 2. Shoaib Raza Rizvi 3. Aftab Ahmed Sheikh 4. Saleem ul Haque

1. Asstt. Prof. of Anatomy, JM&DC, Karachi 2. Senior Instructor, Sind Institute of Urology, Karachi 3. Assoc. Prof. of Anatomy, LCM&D, Karachi 4. Assoc. Prof. of Biochemistry, Avicenna Medical College, Lahore.

ABSTRACT

Background: Noise, whether pleasant or not, is ever present in nature. Not much work can be done without noise; in the industries, fish market, social gatherings; it is constantly in the background. Though it seems harmless loud and or prolonged exposure to noise can cause health problems to the populace. Noise, as a recognized stressogenic factor, has been postulated to stimulate the HPA axis leading to the release of stress hormones.

Aims & Objectives: The present study was designed to assess the changes occurring in the level of stress hormones (ACTH & Corticosterone) in albino rats when acutely (24 hrs) exposed to loud noise with subsequent ameliorating effects of benzodiazepine (Valium/Diazepam) which will given to the rats to alleviate the symptoms of stress.

Study Design: Prospective Experimental Study.

Place and Duration of Study: This study was conducted in the Department of Anatomy, JPMC, Karachi from Jan 2006 to June 2008.

Material and Methods: Three groups of 10 rats labeled A, B and C serving as control (no noise), noise stress (24hrs) and noise stress (24hrs) with diazepam (5mg/kg) respectively. The groups B & C were exposed to white noise at 100dB. At the end of study the animals were sacrificed and their mean plasma concentration of ACTH and corticosterone were measured.

Results: A highly significant increase in mean plasma concentration of ACTH (150.90±14.71pg/ml) & corticosterone (5.72±0.28µg/dl) was observed in noise stressed group when compared with plasma ACTH (53.80±5.75pg/ml) & corticosterone (1.75±0.25µg/dl) concentration of control animals. This increase was significantly ameliorated by pre treatment of diazepam.

Conclusion: Our study shows the protective role of diazepam in stress induced by noise.

Key Words: Adrenocorticotropic hormone (ACTH), corticosterone, Noise stress, Diazepam, Hypothalamic-Pituitary Adrenal (HPA) axis.

INTRODUCTION

Stress is simply a fact of nature and an unavoidable consequence of life. Feeling of stress in humans results from interactions with the environment and is perceived as straining or exceeding their adaptive capacities, and threatening their well being.

Noise is a significant environmental problem [1] and has potential to cause stress reaction [2]. Pakistan is one country where urban sights and sounds resonate more with noise and air pollution than anything else. Air, water and noise pollution level in Pakistan is one of the highest in the world and is causing serious health concerns. According to a study conducted in 1994, noise levels from traffic in Lahore, Karachi, Hyderabad and Faisalabad range from 74-90dbA, 92-94dbA and 73-88dbA respectively. 6 places in Lahore, 7 places in Karachi, 6 places in Hyderabad and 2 places in Faisalabad were identified where the noise levels were greater than maximum permissible levels [3].

Noise level exceeding maximum permissible (60dB day & 50dB night) limits cause several physical ailments and social and emotional problems [4].

Noise, a stressogenic factor, causes disturbances in biochemical parameter of the body [5]. It acts as a physical stressor on animals that can lead to behavioral, physiological and anatomical responses [6]. Studies conducted on rats exposed to loud noise (85dB) have shown to stimulate HPA axis [7, 8].

Noise signals are sub cortically connected via the Amygdala to the Hypothalamic-Pituitary Adrenal (HPA) axis [9]. Studies using single label anterograde and retrograde tracing suggest limited evidence for direct connection between central or medial amygdale and paraventricular nucleus [10, 11, 12].

Noise, as a stressful stimulus is a widely accepted fact. However, an effective agent to counter the noise stress-induced biochemical alteration remains elusive.

Benzodiazepines are the class of drug that is widely prescribed mainly for their anxiolitic and sedative action especially for symptomatic relief of anxiety and tension states resulting from a stressful environmental or emotional factor [13]. As benzodiazepine can counteract the behavioral, neurochemical and hormonal modifications induced by stress, numerous studies have been focused on the role of benzodiazepine receptor in

response to stress. This is not only because of their anxiolitic, anticonvulsive, muscle relaxing and hypnotic properties due to direct action on the central nervous system, but also because of various effects both on non-neuronal tissues including Kidney, Lung, Heart, some endocrine glands and on several peripheral autonomic functions, such as gastric secretion, blood pressure and heart rate [14].

Diazepam, a classical benzodiazepine interacts with the benzodiazepine site of type-1 receptor complex, inhibits serotonin induce CRH secretion [15]. This reduction of CRH secretion leads to highly significant attenuation of the ACTH and cortisol increase following stress [16,17].

The present study was designed to assess the changes occurring in the level of stress hormones (ACTH & Corticosterone) in albino rats when acutely (24 hrs) exposed to loud noise with subsequent ameliorating effects of benzodiazepine (Valium/Diazepam) which will given to the rats to alleviate the symptoms of stress..

MATERIALS AND METHODS

Thirty normal, adult and healthy male albino rats of ages (90 - 120 days) weighing 220-350 grams were used. The animals were kept under observation for one week prior to the commencement of the experiment for assessment of their general state of health on the basis of weight gain or loss. Furthermore, this was also essential to acclimatize the animal to the environment of experimental room. All the animals were kept on standard laboratory diet and under closely controlled environmental conditions of 12 hour light / 12 hour dark cycle at room temperature.

All the animals were divided into three groups. The group A will serve as control, animals of group B were exposed to loud noise100 dB for 24 hours and received injection 0.9% normal saline intraperitoneally before the exposure to noise and the animals of group C were pretreated with inj diazepam (5mg/Kg) before exposure to loud noise 100 dB for 24 hours. The animals were sacrificed at the end of experimental procedure and all the experimental procedures were performed between 8:00 am to 12:00 pm in order to avoid circadian variations.

General appearance, i.e. activity, behavior and their food intake were checked daily.

Noise Stress Induction Procedure: Broad band noise (white noise) was produced by a white noise generator and amplified by an amplifier which was connected by loud speakers installed at a distance of 40cm at two opposite sides of the animal cage. The intensity of the sound was measured by precision sound level meter (TES -1351) Noise level was set at 100 dB uniformly through out the cages. The noise level of 100 dB is chosen because it is comparable with the noise level detected in discos, some industrial places and noise produce by some pressure horns used in most of the metropolitan cities of Pakistan.

Estimation of Plasma ACTH and Corticosterone Concentration: Blood samples were collected (by intra cardiac puncture) in tubes containing 10µl sodium EDTA and kept on ice until centrifuged. After centrifugation, the plasma was aliquoted and kept frozen at -70ºC until assay.

Plasma ACTH was measured by ACTH, RIA kit purchased from Immunotech, France. Standard acylated sample was added to ACTH antibody coated tube and then was incubated for 1 hour at room temperature with shaking. The assay sensitivity was 1.2pg/ml.

Plasma corticosterone was measured by rat corticosterone EIA kit purchased from Diagnostic System Laboratory, USA. The sample was diluted by using sample diluent and was added into antibody coated well, coated by polyclonal rabbit anti-corticosterone antibodies linked to inner surface of polystyrene well. Sample was mixed with enzyme conjugate solution. Then the plates (wells) were sealed and incubated for 24 hours. Later it was washed with wash solution for three times and tetra methyl benzidine was added to all wells and incubated for 30 minutes. Then 0.5 M HCl was added and sample was analyzed using micro plate reader. The assay sensitivity was1.6 ng/ml.

All the results were analyzed statistically by using SPSS version 12, by apply student t test.

RESULTS

Plasma ACTH concentration: Plasma ACTH concentration in various groups was analyzed by using radioimmunoassay kit. Their results and comparison of results are summarized in table 1 figure 1.

A highly significant increase ($P < 0.0001$) in mean plasma concentration of ACTH was observed in animals exposed to acute noise stress for 24hrs (150.90±14.71pg/ml) when compared with mean plasma concentration of ACTH in control animals (53.80±5.75pg/ml).

No significant change was observed in mean plasma ACTH concentration of animals exposed to 24hrs (acute) noise stress with diazepam (67.90±7.12pg/ml) when compared with mean plasma ACTH concentration of control animals clearly indicating the protective effect of diazepam in acute stress induced by noise.

Plasma Corticosterone Concentration: Plasma corticosterone concentration was analyzed in animals of various groups by enzyme immunoassay kit and their observation with comparisons are summarized in table 2, figure 2.

The mean plasma corticosterone concentration in animals exposed to 24 hrs (acute) noise stress was found to be 5.72±0.28µg/dl, which shows highly

significant increase (P <0.0001) when compared with mean plasma corticosterone concentration of control animals (1.75±0.25µg/dl).

Table No.1: Plasma ACTH Concentration pg/ml In Various Groups

Animal No	Control (A)	Acute Noise Stress (B)	Acute Noise Stress + Diazepam (C)
1	65	190	82
2	76	130	44
3	82	208	110
4	34	94	65
5	44	124	62
6	48	230	59
7	53	172	74
8	29	98	38
9	68	135	94
10	39	128	51
Mean	**53.8**	**150.9**	**67.9**
SEM	**5.75**	**14.71**	**7.12**

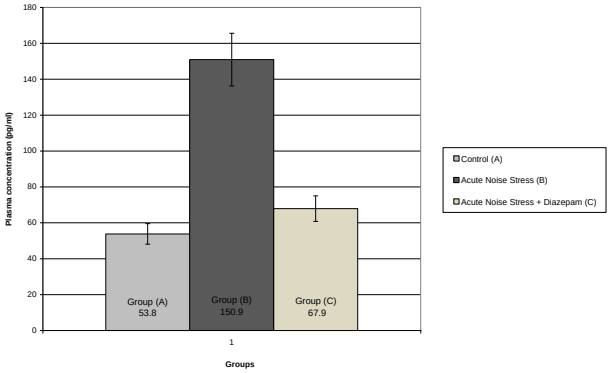

Figure No.1: Plasma ACTH Concentration (ph/ml) in various groups.

Table No.2: Plasma Corticosterone Concentration µg/dl in various Groups

Animal No	Control (A)	Acute Noise Stress (B)	Acute Noise Stress + Diazepam (C)
1	1.3	4.8	1.85
2	1.6	6.7	1.95
3	1.4	5.65	2.25
4	1.26	5.55	2.7
5	1.6	4.95	2.1
6	3.75	7.7	2.25
7	2.25	5.8	1.8
8	0.8	5.75	1.75
9	1.8	5.65	1.65
10	1.7	4.7	1.8
Mean	**1.746**	**5.725**	**2.01**
SEM	**0.25**	**0.29**	**0.1**

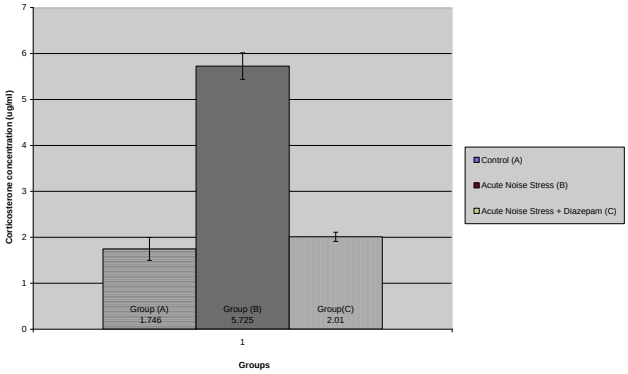

Figure No.2: Plasma Corticosterone concentration (ug/ml)

Insignificant change was observed in mean plasma corticosterone concentration of animals exposed to 24hrs (acute) noise stress with diazepam (2.01±0.1µg/dl) in comparison to mean plasma corticosterone concentration in control animal. This shows the protective role of diazepam in stress induced by noise.

DISCUSSION

Exposure of noise stress provokes cascade of reactions resulting in activation of HPA axis. Activation of HPA axis is the consequence of the convergence of stimulatory inputs from different region of brain into paraventricular nucleus of hypothalamus when the most important ACTH secreatougogues, corticotropin releasing hormone and arginine vasopressin are formed. Plasma levels of ACTH and corticosterone are considered as good marker of stress for three main reasons: (a)Their plasma levels are proportional to the intensity of emotional and systemic stressors, (b) daily repeated exposure to a stressor usually resulted in reduced ACTH response to the same stressor that is termed adaptation or habituation; (c) chronic exposure to stressful situations results in tonic changes in HPA axis that can be used as indices of the accumulative impact of these situations [18].

In our study we have observed the highly significant rise in plasma ACTH and corticosterone concentration when animals were exposed to acute noise stress. Our finding was found in agreement with the observation of Goshen et al., [19] ; Djordjevic et al., [20]; Liebseh et al.,[21] and Zheng and Ariizumi [22].

The Hypothalamus pituitary adrenal axis exerts a variety of effects at both the central and peripheral level. Its activity is mainly regulated by CRH, AVP, and Glucocorticoid.-mediated feedback action [23].

Numerous previous studies has shown that Benzodiazepines profoundly suppress the basal and stress related activation of HPA system and discontinuation of these drugs results in rebound activation[24]. Acute intra peritoneal administration of diazepam (2mg/Kg) inhibits the activity of the HPA axis, i.e., it decreases the concentration of Adreno-corticotropic hormone (ACTH) and corticosterone in female rats [25].

Benzodiazepine, possess a clear inhibitory influence on the activity of HPA axis in both animals and humans. This effect seems to be mediated at the hypothalamic and/ or supra hypothalamic levels via suppression of CRH [17,26,27]. A number of experimental studies clearly suggest that benzodiazepines attenuate the CRH secretion possibly through inhibitory GABAergic neurons [16,28].

In animals treated with diazepam before exposure to acute noise stress significant decrease in the plasma level of ACTH and corticosterone was observed. Several studies support our observations. Fukumistu et al., (2005)[29] found significantly lower serum corticosterone concentration in animals treated with diazepam (10mg/Kg) before subjected to psychological stress. Eisenberg [30] observed a significant protection of pretreatment of diazepam (5mg/Kg) from rise in plasma corticosterone concentration in animals exposed to sound vibration, a non invasive stress. According to Rohrer et al.,[16], administration of benzodiazepine led to a highly significant attenuation of ACTH increase following stress.

CONCLUSION

Our study shows the protective role of diazepam in stress induced by noise.

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Address for Corresponding Author:
Syed Meesam Iftekhar. H. Rizvi,
Asstt. Prof. of Anatomy,
Jinnah Medical & Dental College, Karachi.
B-256 Sector 11-B North Karachi,
Cell No: 0300-2076129
E-mail:i_m_meesam2004@yahoo.com