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THE EFFECT OF ACUTE NOISE STRESS ON NEUTROPHIL FUNCTIONS

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Abstract : The effect of acute noise stress on albino rats was studied by estimating the plasma corticosterone level, total leukocyte count and differential leukocyte count. Neutrophil function was assessed by Candida phagocytosis and Nitroblue tetrazolium reduction test. The total leukocyte count was significantly decreased. No significant changes were observed in the differential count of the leukocytes. A significant increase in the plasma corticosterone level, Candida phagocytosis and Nitroblue tetrazolium reduction was observed indicating acute noise to be a potent stressor in albino rats.

Key words : noise stress, corticosterone, leukocyte, neutrophil function

INTRODUCTION

Stress is the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects. In our daily lives, some stress prepares us to meet certain challenges. The productive stress is called Eustress while the other harmful stress is called Distress. If the stress is extreme the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Under these conditions stress triggers a wide range of body changes called General Adaptation Syndrome (GAS) (1). The stimuli, which produce GAS, are called the stressors.

Noise is the most wide spread form of environmental stressor in the industrialized urban areas. Noise is defined as any unwanted sound that may adversely affect the health and well being of individuals or population (2). The extent of the discomfort experienced by an individual, subjected to noise will depend upon the frequency spectrum, intensity of the sound, duration, aural sensitivity of the listener, and upon the activities being undertaken at the time of noise exposure.

Acute exposure to noise stress can lead to deafness. People exposed to sound levels exceeding 80 dB for 8 hours/day show hearing impairment (3). Patients exposed to 30 minutes noise stress (105 dB) had

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elevation of blood pressure (4). Studies have shown that exposure to high noise levels near major airports may cause increased incidence of birth defects in the offspring of parents residing near these airports (5). Increase in the plasma levels of adrenaline, noradrenaline and corticosterone has been observed in rats exposed to acute noise stress (6). It is clear that noise stress produces widespread disturbances in the biochemical and physiological activities of human beings and animals.

Though extensive studies have been done on the extra auditory effects of noise stress, very little literature evidence exists on the acute effect of noise on neutrophil functions. As all of us are exposed to noise in our day to day lives, we found it highly relevant to study the effect of acute noise stress on neutrophil functions and haematological parameters.

METHODS

Wistar strain male albino rats weighing 180–200 g were used for the study. Rats were housed under standard laboratory conditions with food and water provided *ad libitum* and were divided into two groups. Control group: This group of rats (n=15) were kept under standard laboratory conditions and Noise stress group: This group of animals (n=15) were subjected to white noise of 100 dB intensity for 4 hours and sacrificed after 30 minutes.

Noise stress procedure

White noise was produced by a white noise generator (Graphic-Pilot) and amplified by an amplifier which was connected to a loudspeaker located 30 cm above the animal cage. The intensity of the sound was measured by a sound level meter (Cygnet-D 2023). The background noise level inside the room was 42 ± 3 dB, produced by the ventilation system.

The animals were anaesthetized with ether rapidly within 2 minutes according to the stress free procedure of Feldman and Conforti (7) which does not cause stress to the animals as evidenced by stable corticosteroid level in blood. This procedure also causes no change in the hematological parameters studied in this investigation. The blood sample was collected from the jugular vein immediately after anaesthetization and was used for the estimation of total leukocyte count, differential count, neutrophil function tests and plasma corticosterone.

The total and differential leukocyte counts were estimated by standard methods. The phagocytic ability of the neutrophils was studied by noting the Candida phagocytosis (8). To leukocyte suspension obtained from the buffy coat of 0.5 ml heparinized blood, heat killed candida albicans was added and incubated at 37°C for 15 min and centrifuged. Smears were made using the sediment and stained with Leishmans stain. This test relies on the uptake of heat killed candida albicans by phagocytes over a brief period of time. The intracellular candida, stain intensely and can be identified and counted inside the neutrophils under oil immersion. The number of neutrophils positive for candida ingestion in 100 neutrophils gives the Phagocytic index (PI) and the total number of candida albicans counted within 100

positive cells divided by 100 gives the avidity index (AI). The killing ability of the neutrophils was assessed by the Nitroblue tetrazolium (NBT) reduction test (9). Granulocytes were isolated by incubating 0.5 ml of heparinized blood over a glass slide at 37°C for 30 min. The blood was washed and to the neutrophils adhered to the slide was added NBT and incubated at 37°C for 30 min. The slide was fixed, stained and counted under oil immersion. When neutrophils are exposed to the yellow dye NBT, it is taken up by the cells into phagosome and intracellular reduction of the dye converts it to insoluble blue formazon crystals. 200 cells were observed and the positive cells for formazon were counted and expressed as percentage of positive cells. The plasma corticosterone level was estimated by the spectrofluorimetric method (10).

Statistical analysis was done by the Student's t test.

$P<0.05$ was considered statistically significant.

RESULTS

The total leukocyte count was significantly decreased in the noise stress group ($P<0.001$). No variable differences were observed in the differential count of the leukocytes (Table I).

A significant increase in the Plasma corticosterone level ($P<0.001$) was observed in the stressed group. The phagocytic index ($P<0.001$) and the avidity index ($P<0.001$) were significantly raised in the stressed animals when compared with the control animals. The Nitroblue tetrazolium reduction was significantly raised ($P<0.001$) in the noise stress group (Table II).

TABLE I : Total leukocyte count and differential count in acute noise stress.

Parameter	*Control (n=15)*	*Noise stress (n=15)*
Total leukocyte count (per cu.mm)	14155±2316	7630±1871
Differential count (%)		
Lymphocyte	69.4±2.2	69.5±2.5
Neutrophil	21.3±1.7	20.4±3.1
Monocyte	9.3±2.1	9.5±1.5
Eosinophil	0.5±0.5	0.6±0.5
Basophil	0.4±0.5	0.2±0.4

Values are expressed as Mean ± SD; Significance: *P<0.001.

TABLE II : Plasma corticosterone and neutrophil function tests in acute noise stress.

Parameter	*Control (n=15)*	*Noise stress (n=15)*
Plasma corticosterone (µg/dl)	42.8±4.3	95.9±9.6*
NBT (%)	9.6±1.5	16.3±2.2*
Phagocytic index	68.3±2.9	85.3±3.7*
Avidity index	2.6±0.3	3.7±0.2*

Values are expressed as Mean ± SD; Significance: *P<0.001.

DISCUSSION

The leukopenia observed in our study is similar to the recent report (11) and it could be due to the excessive release of corticosteroid in stress, which was known to cause leukopenia.

The differential count of the leukocytes did not show any significant variation. This

is contrary to the previous reports in which stress-induced reduction in lymphocyte count (12) and eosinopenia (13) has been reported. However, previous studies in our laboratory in cold swimming stress (14) on differential count have shown no significant variation in the lymphocyte and neutrophil count. Recent work of Van Raaij et al (15) has also shown no variation in differential count after noise stress. Probably this contradictory result in differential count in stress could be due to the complex interplay of various physiological factors, the balance of these agents will determine the out come of the numbers of the circulating different white blood cells. Though the duration and nature of these experiments are not directly comparable with those of this study, it may be safely stated that other factors like neurotransmitters, pituitary peptides and adrenal hormones are the physiological mediators for the changes observed.

A significant enhancement in the neutrophil functions has been observed in our study. The killing ability of the neutrophils as indicated by the NBT reduction and the phagocytic ability of the neutrophils as indicated by the phagocytic index and avidity index has been significantly increased in the noise stressed animals. Scanty literature evidence exists on neutrophil function tests in stress. However, reports have shown that phagocytosis is an energy-mediated phenomenon and cyclic adenosine monophosphate (cAMP) acts as a second messenger. cAMP regulates the selective extrusion of lysosomal enzymes in phagocytosing neutrophils and ability to kill candida albicans. The phagocytic cells contain alpha and beta-adrenergic receptors which when stimulated can elevate cellular cyclic guanosine monophosphate (cGMP) whereby an alteration in the endocrine environment or autonomic output could modulate the neutrophil function. Sympathetic neurohormones (catecholamines) acts through cAMP, while parasympathetic neurotransmitters (acetylcholine or its conjugates) act through cGMP (16). The changes observed in neutrophil function in this study may be due to the interaction of these different factors.

In addition, leukocytes possess glucocorticoid receptors and membrane receptors to mediators like Adrenocorticotropic hormone (ACTH) and Corticotropin releasing factor (CRF). In our study an increase in corticosteroid level has been observed which could have mediated the changes in the neutrophil function via the receptors present on the leukocytes. Studies in mice exposed to acute noise stress have shown similar increase in oxidative response of the peritoneal macrophages. Phagocytes have beta adrenergic receptors and possess receptors for neuropeptides. Thus phagocytes can be greatly affected by the nervous system products. So, the phagocytic and oxidative response of neutrophils may be controlled by the sympathetic nervous system (17).

The significant elevation in the plasma corticosterone level observed in the noise stress group could be due to norepinephrine acting at CRF neurons in the paraventricular nucleus to directly stimulate CRF release and subsequently ACTH and corticosterone secretion, in noise exposure (18). Thus our findings are in

agreement with the previous study of Sandi et al (19) who have shown enhanced ACTH and corticosterone levels in rats exposed to noise stress. Further our recent studies have also shown an increase in plasma corticosterone level in rats exposed to acute noise stress (20).

From our studies it is clear that acute exposure to white noise causes various physiological changes like leukopenia, a significant elevation in the corticosterone level and an enhancement of the neutrophil functions thus acting as a potent stressor.

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