

Effects of Noise Exposure on the Volume of Adrenal Gland and Serum Levels of Cortisol in Rat

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Abstract

Background: Noise pollution is considered as a stressful factor in every day's life of the industrial world. The present study was designed to investigate the probable effects of different time exposure of noise stress on histomorphometric changes of adrenal gland, plasma cortisol and body weight of the rat.

Methods: Sixty adult male Wistar rats were divided into two series of short time noise or STNE exposure (n=20) for one day and long time noise exposure or LTNE (n=40) for 30 days with a sound intensity of 100 dBA. LTNE series were further divided into five groups of control, sham, 4, 8 and 12-hrs noise exposure and STNE series were divided into four groups of the same as LTNE except sham group. The cortisol assay was performed on blood samples of pre and post treatment with noise in two series and Cavalieri methods in a stereological study for determination of adrenal volume changes only in LTNE series.

Results: The results showed that the mean plasma cortisol level (5.4 ± 3.2 $\mu\text{g/dl}$ in LNTE-8hrs and 4.9 ± 1.8 $\mu\text{g/dl}$ in LNTE-12hrs), the volumes of cortex (7.08 ± 1.31 mm^3 in LNTE-8h and 7.12 ± 1.31 mm^3 in LNTE-12h), medulla (0.68 ± 0.19 mm^3 in LNTE-8h) of adrenal gland increased. The mean animal weight did not show any significant changes.

Conclusion: Sound pollutions can increase the stress hormone level and cause increasing the volume of adrenal gland that may be related to the effect of noise on the hypothalamus-pituitary-adrenal axis. These results may be important to increasing of many disorders such as cardiovascular diseases in industrial societies.

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Keywords • Adrenal gland • volume • cortisol • noise exposure • stress

Introduction

Stress can be defined as a state of threatened balance, induced by external stressor and appear as the display of somatic and psychic reactions, struggling to regain homeostasis.¹ Among stressful stimuli, noise is an environmental pollutant capable of causing hearing impairment,² behavioral, mental and widespread disturbances at several levels in human organs and apparatus due to chemical and physiological

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modifications of endocrine, cardiovascular and nervous systems.³ It has been hypothesized that the connection between thalamic structures of the auditory system and subcortical areas, such as amygdala, hippocampus and hypothalamus act as a fast reaching memory chain, which establishes and enhances adverse excitation during noise exposure.⁴ The sound-evoked excitations reach hypothalamic-pituitary-adrenal system (HPAS) causing a subsequent rise, via corticotrophin-releasing hormone, in the levels of corticotrophin and the corticosterone.⁴

In recent years, the measurement of stress hormones including adrenaline, noradrenaline and cortisol have been under consideration in noise-exposed subjects of cardiovascular risk factors.⁵ Animal experiments showed that noise changed the sensitivity of cellular cortisol receptors by an increase in heat-shock protein and in ultra structure in heart and adrenal glands.⁶ Many stereological studies have also shown that the volumes of zona fasciculata and zona reticularis of adrenal cortex were increased with aging,^{7,8} ethanol consumption,⁹ and administration of oxytocin.¹⁰ However, quantitative study of the influence of loud noise on the adrenal gland has received little attention. Therefore the present study was designed to investigate the probable effects of chronic exposure of noise stress on adrenal gland.

Materials and Methods

Animals and stress protocol

Experiments were performed in conformity with the university research council guidelines for conducting animal studies. Sixty adult male Wistar rats (250-300 g) were housed in open wire cages in a temperature-controlled room (22-24°C) and 12 hrs light-dark cycles with free access to standard laboratory rat chow and tap water. In order to minimize all undesired stressors, such as handling, habitat, etc, animals were not exposed to noise for at least one week after delivery to the laboratory.

The experiments were performed in two series including a long-time and a short-time noise exposure. The long-time noise exposure series included five groups comprising sham, control and three groups receiving various durations of noise exposure. The sham group was transferred to a sound room when the noise generator was off and the environmental noise was less than 10 dBA. The control group was kept in the animal room, without being transferred to the sound room. The three noise-exposed groups (n=8 each) were placed in sound room and for 30 consecutive days were kept in noise room and received noise exposure for 4 hrs (from 16:00 to 20:00), 8 hrs

(from 20:00 to 4:00) or 12 hrs (from 4:00 to 16:00) respectively. Noise exposure was performed using a noise generator by playing-back a recorded noise at 100 dBA, in cages using a sound level meter (Lutron, Taiwan). In the animal room, all groups were exposed to a noise with an intensity of approximately 40 dBA, related to fan and other environmental sounds. The short-time series consisted of control and three groups of rats (n=5 each) which were exposed for one day to noises with a similar intensity and durations as of long-time exposure series, without considering sham group.

Cortisol assay

In the long-time exposure series, the cortisol levels were measured twice on day 1 (pre-exposure measurement) and on day 30 (post-exposure measurement). To collect pre-exposure blood samples of LTNE series, animals were anesthetized using diethyl ether, and their tails were cleansed using warm water. Samples were withdrawn by puncturing lateral or ventral tail vein with a needle (gauge 23) connected to a piece of heparinized cannula. Post-exposure blood samples were withdrawn through a dorsal aortic puncture under anesthesia using anesthetic ether. Blood samples were centrifuged (2000 g) and their serums were separated and frozen. Serum levels of cortisol were determined by radioimmunoassay solid phase using cortisol labeled with I₁₂₅ (DPC company; USA).

Tissue processing and sampling

Rats of long-time noise exposure series were sacrificed under deep anesthesia, with an overdose of anesthetic ether. The whole adrenal gland was removed quickly and fixed in buffer formalin solution (4%) for one week, and then paraffin blocks were prepared.¹¹ From each block 7-10 systematic random sections (7 µm thick) were stained with H&E.^{12,13} The image of each section was taken, using a digital camera (Moticam model 350, Japan) placed on a light microscope with constant magnification, and then transferred to a PC computer. Morphometric study was done using stereological software which was a transparent test system composed of 900 points. Cavalieri method was used for the determination of the cortex, medulla and total volume of the adrenal gland.^{14,15} The area at the level of the object was confined by the cordless natural pen device and the points hitting the object transect were counted. For determination of inter-point spacing of the point grid, the linear magnification was measured using the image of standard 0.01 µm graticule (Zeiss; Germany) with similar magnifications for all images.

$$CE_{(v)} = \frac{1}{\sum_{i=1}^m p} \left(\frac{1}{12} (3a + c - 4b) \right)^{1/2}$$

$$a = \sum_{i=1}^m p_i \cdot p, \quad b = \sum_{i=1}^{m-1} p_i \cdot p_{i+1}$$

$$c = \sum_{i=1}^{m-2} p_i \cdot p_{i+2}$$

when CE is <25% (0-10%), the results are considered as valid and when CE>25% they are considered invalid.

The coefficient error (CE) of the Cavalieri method for each block was calculated using the following equation.¹⁵

Statistical analyses

Data are presented as mean±SD. The volumes of adrenal gland in the long-time noise exposure series were compared with that of control and sham groups separately using Mann-Whitney-U test. The serum levels of cortisol in different groups of STNE and LTNE series were analyzed by one way ANOVA followed by Tukey test and the pre- and post-exposure levels of cortisol in different groups of LTNE series and animals body weights were analyzed using paired Students t-test, and $P<0.05$ was considered as statistically significant.

Results

The serum levels of cortisol from short-time and long-time noise exposure series are shown in Table 1. In short duration of loud noise exposure no significant differences were noticed in relation to this stress hormone between control, sham and exposures groups. The pre- and post-exposure serum levels of cortisol in 8 hrs and 12 hrs noise exposed (NE-4h, NE-8h) of long-time-exposure series (Table 2) are significantly different from control or sham groups. Whereas, the serum levels of cortisol of control, sham and 4 hrs noise exposure groups (NE-4h) were not significantly different from each other (Table 2). The serum cortisol levels of long-time-exposure (8-h and 12-h) groups were significantly different from that of control and sham groups (post-exposure, Table 1).

No changes were noticed in the histological appearance of the adrenal tissue in all of groups. The volumes of the adrenal glands increased significantly in groups exposing to noise for 8 or 12 hrs with compared to control and sham groups. The volume of adrenal glands in the sham-exposed group decreased compared to control but was not significant and was not confirmed by statistical analysis. The CE of Cavalieri method was calculated between 3% and 10%. There was no signifi-

cant difference among the first (day-0), middle (day-15) and the final (day-30) weights of rats in long-time noise exposure (Table 3).

Table 1: Serum levels (mean±SD) of cortisol (µg/dl) in short-time (STNES) and long-time noise exposure series (LTNES) of control, sham, and noise-exposed rats (NE) at the onset (pre-exp) and at the end (post-exp) of the experiments.

groups	cortisol (µg/dl)		
	STNES	LTNES	
		pre-exp	post-exp
control	2.0±0.2	1.6±0.6	1.1±0.5
sham	-	1.4±0.8	1.3±0.7
NE-4 h	2.7±1.5	1.8±1.6	3.3±3.1
NE-8 h	2.4±2.2	2.4±0.8 □	5.4±3.2 □*
NE-12 h	2.8±1.4	2.2±1.5 □	4.9±1.8 □*

* Significantly different from control ($p<0.05$)

□ Significantly different from pre- and post-exposed groups ($p<0.05$)

Table 2: The volumes (mean±SD) of cortex, medulla and total adrenal gland (Cavalieri method) of control, sham and LTNE series exposed to noise (NE) for 4, 8 or 12-h.

groups	cortex (mm ³)	medulla (mm ³)	Total (mm ³)
control	5.35 ± 0.78	0.42 ± 0.15	5.76 ± 0.88
sham	5.23 ± 1.22	0.35 ± 0.13	5.57 ± 1.27
LTNE-4h	6.44 ± 1.17	0.52 ± 0.12	6.90 ± 1.28
LTNE-8h	7.08 ± 1.31*§	0.68 ± 0.19*§	7.79 ± 1.46*§
LTNE-12h	7.12 ± 0.58*§	0.48 ± 0.21	7.62 ± 0.70*§

* Significantly different from control group at $p<0.01$

§ Significantly different from sham group at $p<0.01$

Table 3: The values (mean±SD) of body weights (g) of rats in the control, sham and long-time noise exposure for 4, 8 or 12-h groups at the start (day-0), and after 15 and 30 days of exposure (day-15, day-30).

groups	Day-0	Day-15	Day-30
Control	262±23	304±37	318±35
Sham	257±28	285±40	293±45
NE-4h	270±15	305±23	311± 24
NE-8h	257±36	282±26	289± 23
NE-12h	273±29	279±23	291± 22

Discussion

This study demonstrated a significant alteration in adrenal gland when loud noise was applied, an important problem of our industrial society and today life. The plasma concentration of cortisol increased in 8 hrs and 12 hrs long time noise exposed group at day 30 compared to day 0 and also with the cortisol concentration of the control and sham-exposed group that may be due to responses of HPA axis to stress.¹⁶ The study on workers exposed to noise, showed an increase in heart rate and in norepinephrine and cortisol levels.¹⁷ A significant increase in plasma corticosteroid level was reported in albino rats.¹⁸ Other researchers have reported higher cortisol or corticoid concentrations after chronic exposure to noise stress in human and animals.^{6,19,20} It has been demonstrated that noise-induced cortisol

secretion based upon the existence of very closed connection between subcortical structure of central nervous system and part of auditory system can activate HPA axis. The increased volumes of cortex, medulla and total adrenal gland in 8 hrs and 12 hrs noise-exposed groups may be also correlated to the hyperactivity of HPA axis causing an increase in the activity and secretion of this gland. The hyperactivity of adrenal gland may be dependent on cell hypertrophy or organelle hyperactivity or hypertrophy. After administration of ACTH in hamsters, a marked increase was observed in adrenal gland weight and enlargement of the zona fasciculata cells due to a notable increase in the volume of mitochondria, Smooth endoplasmic reticulum, Golgi apparatus and lipid droplet compartment.²¹ Some stereological studies have shown hypertrophic changes in different layers of adrenal cortex using other stresses or chemical agent.⁷⁻¹⁰

Conclusion

Environmental sound pollution as a stressor seems to increase plasma cortisol via adrenal hyperactivity and hypertrophy as a result of cellular hypertrophy or cellular hyperplasia.

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