EFFECT OF DIAZEPAM ON THE SUPRARENAL CORTEX OF ALBINO RAT EXPOSED TO NOISE STRESS. AN ELECTRON MICROSCOPIC STUDY

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ABSTRACT

This study aimed to demonstrate the effect of diazepam administration on the noise-induced ultra structural alterations of rat adrenal cortex.

Adult albino rats of both sexes were used in this study. The animals were divided into 3 groups; control group, noise - exposed group (6 hours daily for 14 days) and diazepam-intra-peritonealy injected group (0.5 mg \100gm body weight, 30 minutes prior to noise-exposure). Assay and statistical analysis of serum cortisol in addition to electron microscopic study of ultra-thin sections of adrenal glands were done.

In the control group, the serum level of cortisol was found to be 160±7.6 ng/ml. Electron microscopic examination of the zona glomerulosa cells revealed elongated mitochondria with lamellar cristae. The zona fasciculata cells exhibited large amount of lipid droplets and plentiful smooth endoplasmic reticulum, the mitochondria were spherical in shape with densely packed vesicular cristae. The cells of the zona reticularis revealed less lipid droplets, more mitochondria, which were rounded or spherical with densely packed tubulo-vesicular cristae.

After exposure to noise stress, the serum level of cortisol was significantly increased. The cells of zona glomerulosa showed no structural

changes apart from the appearance of some round mitochondria containing a mixture of lamellar and vesicular cristae. Mitochondria in some zona fasciculata and zona reticularis cells showed cristolysis and some giant mitochondria were detected and the smooth endoplasmic reticulum tubules were dilated. The zona reticularis cells revealed some bizarre shaped mitochondria with mitochondrial membrane rupture. Some nuclei appeared shrunken with condensed chromatin.

On the other side, serum cortisol level of noise-stressed rats pretreated with diazepam was significantly decreased to about the normal level. No detectable ultra-structural changes were seen in the suprarenal cortex.

It could be concluded that, the function and the structure of the suprarenal cortex were affected by exposure to noise stress. These changes became markedly reduced by diazepam pre-noise administration. Therefore, administration of diazepam in stress conditions is recommended in order to protect the adrenal gland from the effect of increased secretion of adrenocoricotrophic hormone (ACTH).

INTRODUCTION

Exposure of rats to stress stimuli was found to cause considerable increase in the activity of pituitary-adrenocortical axis leading to release of ACTH which in turn stimulates zona fasciculata leading to increase plasma level of cortisol (Axelrod & Reisine, 1984 and De Boer & van der Gugten, 1989).

This hormone -with others- serve to adapt the body to a variety of stressors ranging from psychological forms as fear and anxiety to physical forms like heat and cold (Axelrod & Reisine, 1984).

Most of studies concerning the effect of noise on adrenocortical gland have been focused on measuring functional behavioral and biochemical changes (Borrel et al., 1980; Alario, 1987). Diazepam acts at central level exhibiting anxiolytic property (Ninan & Insel, 1982). Diazepam treatment has been found to reverse the effect of different stressful stimuli (Andrew & File, 1992) and reduce the ultrastructural changes induced by noise exposure on rat's adrenal cortex (Pellegrini et al., 1998). Recently, ultrastructural changes in rat's adrenal gland following exposure to loud noise for a brief period of time was evaluated (Soldani et al., 1999 and Gesi et al., 2001).

Although many studies have reported the effect of diazepam on biochemical changes caused by noise on adrenocortical gland, little information is available concerning the ultrastructural study.

The aim of this work is to study the effect of diazepam administration on the noise- induced ultra structural alterations of rat adrenal cortex.

MATERIALS AND METHODS

Twenty four adult albino rats of both sexes (weighing about 150-200gm) were used throughout this study. The rats were divided equally into 3 groups; control group, noise-exposed group (6hours daily for 14 days through a loud broadcast radio station) and diazepam-injected group (0.5 mg/100gm body weight diazepam {Merk} intra-peritonealy, 30 minutes before exposure to noise).

The animals were isolated in separate cages (two animals /cage to avoid stress by overcrowding). Also, female rats were isolated from males to avoid sexual excitement. The animals were fed on laboratory food and water ad-libitum.

At the end of the experiment, the animals were anaesthetized by intramuscular injection of ketamine (90mg/kgm). Blood samples were aspirated trans-cardially for assay of serum cortisol by immunolite quantitative technique. The suprarenal glands of each animal were dissected carefully and 1 mm^3 thick specimens were fixed in a mixture of 2.5% glutaraldehyde and 2.5 % paraformaldehyde in 0.1 M in cacodylate buffer for 24 hours, and then post-fixed in osmium tetroxide for 24 hours at 4 C^o followed by rinsing in cacodylate buffer for 20 minutes. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in propylene oxide for 30 minutes, and then they were placed in propylene oxide : epoxy resin (1:1) and left overnight, then placed in epoxy resin for another night. Embedding was in absolute resin. Semi-thin sections were stained with 1% toluidine blue to locate the sites of interest, and ultrathin sections were cut and picked on copper grids and stained with uranyl acetate and lead citrate (Hayat, 1989). Sections were examined at Ain-Shams Specialized Hospital, using Zeiss 100s transmission electron microscope operated at 60 KV.

Data Analysis :

* Statistical analysis of data was done by using Excel program and SPSS program (Statistical Package for Social Science version) on windows XP.
* KS test (Kolmogrove Sminrov) was used to test the normality of data distribution which revealed to be normally distributed.
* Data description was done in the form of mean \SD (quantitative data).
* Data analysis was done to test statistical significant difference among groups.
* For quantitative data (mean ±SD):

- One way ANOVA test was used to compare more than two groups followed by Student t-test to compare between each two groups.

NB : P is significant if ≤ 0.05 at confidence interval of 95%.

RESULTS

I) General Behavior of the Experimental Rats :

Stressed animals were irritable and showed a decrease in their food intake, while diazepam-pretreated stressed animals were calm and their food intake was almost like that of the control group.

II) Assay of serum cortisol :

Table (1) and Graph (1): show the serum cortisol level in the studied groups. It was 160±7.6 ng\ml in control group, 450±6.3 ng\ml in stressed rats while in stressed rats pretreated with diazepam it was 168 ±7.5 ng\ml.

There was statistically ,significant increase ($P < 0.05$) in the cortisol level in the stressed group as compared with the control groups , and there was statistically significant decrease ($P < 0.05$) in the cortisol level in the stressed rats pretreated with diazepam as compared with the stressed group. However, there was insignificant increase ($P > 0.05$) in the cortisol level in stressed rats pretreated with diazepam as compared with the control group.

Table (1): Cortisol levels of the studied groups.

	No	Mean ng/ml±SD	F	t	P
*Control rats	8	160±7.5994	1277.214		
*Stressed rats	8	450±6.3421		-38.716*	0.000
*Stressed rats pretreated with diazepam.	8	168±7.5057		-1.491# 46.065@	P = 0.158 P =0.000

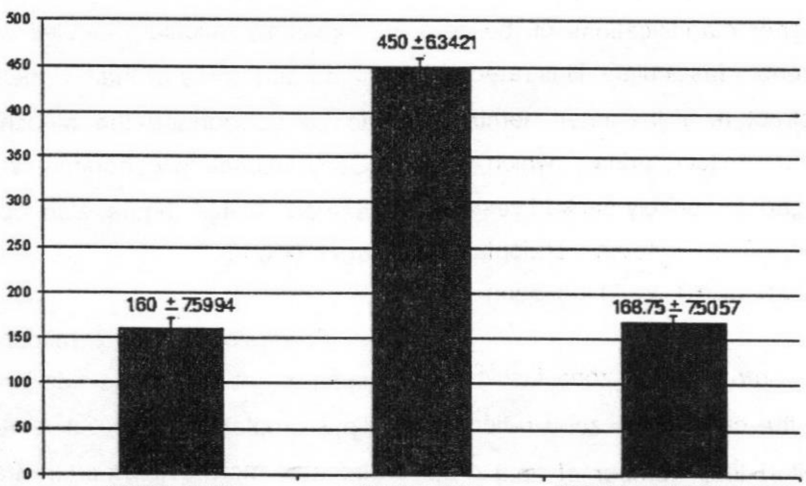
Comparison done through one way ANOVA test followed by Student t test for comparison between each two groups. P< 0.05= Significant. P> 0.05= Non-significant.

*** Significant in comparison to control.**

 # Insignificant in comparison to control.

@ Significant in comparison to stressed.

Graph - 1: Cortisol Levels of the Studied Groups



III) Electron Microscopic Examination :

A) Control Rats :

Examination of the zona glomerulosa cells revealed vesicular nuclei and elongated or oval mitochondria with cristae arranged in lamellar manner (Fig. 1).

Zona fasciculata cells exhibited larger amount of lipid droplets than the other zones. These lipid droplets appeared non-membrane bounded and variable in size. The nucleus was rounded, vesicular with prominent nucleolus. The mitochondria were almost spherical in shape and variable in size (Fig. 2).

Higher magnifications of the cells of zona fasciculata illustrated the fine structure of the inner membrane of the mitochondria , which was arranged in densely packed vesicular cristae. The smooth endoplasmic reticulum (sER) could be seen (Fig. 3).

In contrast to the zona fasciculata cells, the cells of the zona reticularis exhibited less number of lipid droplets, mitochondria were more numerous, rounded or spherical with densely packed tubulo-vesicular cristae (Fig. 4).

B) Stressed Rats :

After noise stress, zona glomerulosa cells showed no morphological changes, apart from some mitochondria became rounded and contained mixture of lamellar and vesicular cristae (Fig. 5).

Some zona fasciculata cells showed striking changes mainly at the mitochondrial level. Some mitochondria showed cristolysis where they contained loosely packed vesicular cristae (Fig.6). Lipid depletion was detected (Figs. 7&8). The smooth endoplasmic reticulum tubules were dilated and some of them appeared as to be surrounded the mitochondria. Conventional mitochondria were observed. Myelin figure also could be seen (Fig. 8).

Protrusion from the mitochondrial inner membrane at the side facing the lipid droplet could be detected and became more evident after stress exposure. Some giant mitochondria could be detected and showed cristolysis (Fig. 9).

The zona reticularis cells showed changes like to that present in the zona fasciculata, where some mitochondria showed cristolysis with less dense matrix. Others showed mitochondrial membrane rupture with non-homogenous cytoplasm. While conventional mitochondria might be present. The changes in the zona reticularis were not restricted upon the mitochondria only but also the nucleus was affected. The nucleus in some cells of the zona reticularis appeared shrunken with condensed chromatin (Fig. 10).

In other cells, the mitochondria became gigantic with cristae organized in parallel manner. Dilated sER also were detected. Some mitochondria became bizarre shaped and autophagic vacuoles containing degenerated mitochondria were also seen (Figs. 11&12)

C) Stressed Rats Pretreated With Diazepam :

Compared with the control suprarenal cortex, examination of zona glomerulosa of the suprarenal cortex of stressed rats pretreated with diazepam by EM showed no morphological changes. Nearly all the mitochondria were oval or elongated with lamellar cristae (Fig.13).

In the zona fasciculata cells of stressed rats pretreated with diazepam showed similar ultra-structure of control rats (Fig.14).

E\M examination of the zona reticularis cells of stressed rats pretreated with diazepam revealed no morphological changes as compared to the control animals. The mitochondria were rounded with tubulo-vesicular cristae. The nucleus was rounded and vesicular. The lipid droplets were less than those of the zona fasciculata cells (Fig.15).

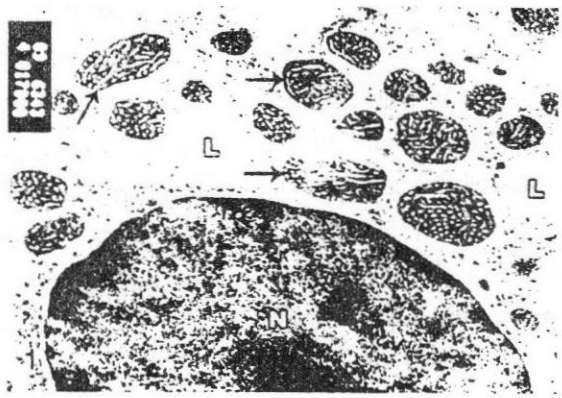


Fig (1) : An electron micrograph of a zona glomerulosa cell of the suprarenal cortex of adult control rat. The vesicular nucleus (N) can be seen. Rounded, oval or elongated mitochondria (arrows) are present. Notice the lamellar appearance of the inner mitochondrial membrane. Lipid droplets (L) are also seen. (TEM x 17,000)

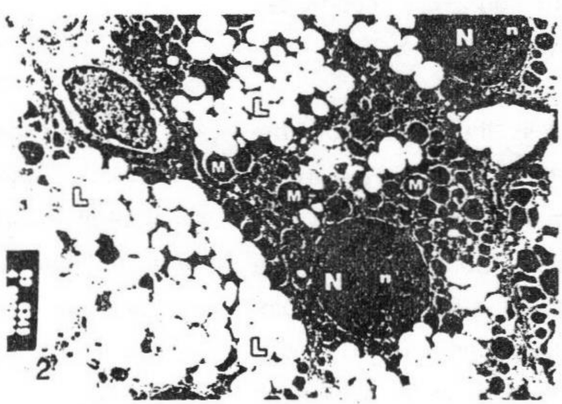


Fig (2) : An electron micrograph of a section in the zona fasciculata of the suprarenal cortex of adult control rat, showing zona fasciculata cells. The nucleus (N) is vesicular with prominent nucleolus (n). The mitochondria (M) are numerous, almost spherical in shape and variable in size. Plenty of lipid droplets (L) are observed. The lipid droplets are non-membrane bounded, variable in size and have electron lucent content. (TEM x 2,800)

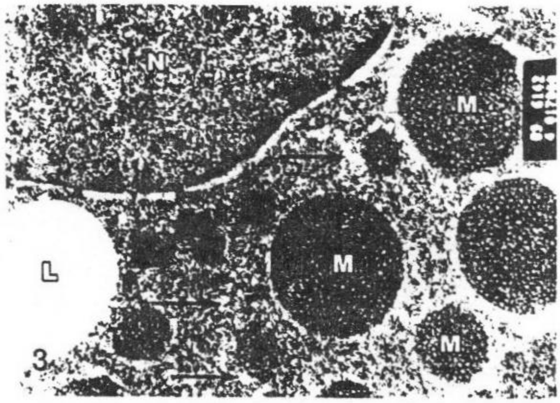


Fig (3) : An electron micrograph of a zona fasciculata cell in the suprarenal cortex of adult control rat showing the vesicular nucleus (N) with its prominent nuclear pores. The spherical mitochondria (M) are variable in size with densely packed vesicular cristae. SER tubules (arrows) and a non-membrane bounded lipid droplet (L) with electron-lucent content are seen. (TEM x 17,000).

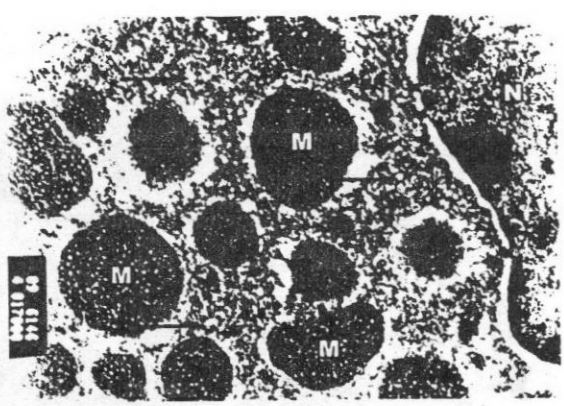


Fig (4) : An electron micrograph of a zona reticularis cell in the suprarenal cortex of adult control rat. Note the vesicular nucleus (N) with its nuclear envelope. Spherical mitochondria (M) can be seen with densely packed vesicular or tubulo-vesicular cristae. SER tubules (arrows) are seen. Notice the proximity between SER & mitochondria. (TEM x 17,000)

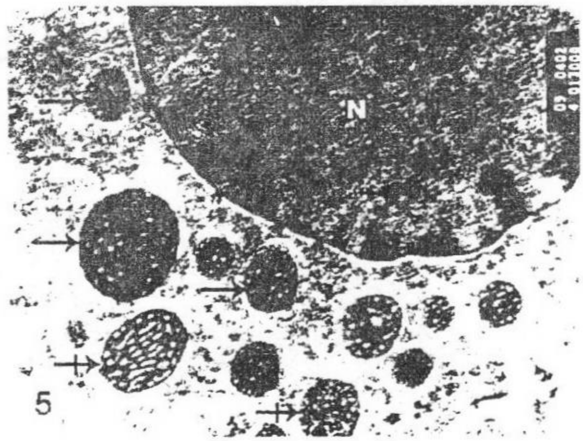


Fig (5) : An electron micrograph of a zona glomerulosa cell in the suprarenal cortex of stressed rat. The nucleus (N) is vesicular but with more condensed peripheral chromatin than that of the control. Some mitochondria become spherical with vesicular cristae (arrows). Others conventional elongated mitochondria with lamellar cristae can be seen (crossed arrows). (TEM x 13,000)

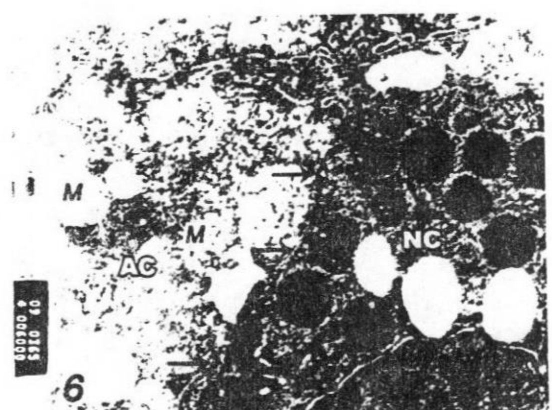


Fig (6) : An electron micrograph of a section in the zona fasciculata of the suprarenal cortex of a stressed rat showing an intact cell (NC) and affected cells (AC) containing some mitochondria (M) with loosely packed vesicular cristae. Notice intercellular digitations (arrows) among the adrenal cells. (TEM x 6,000)

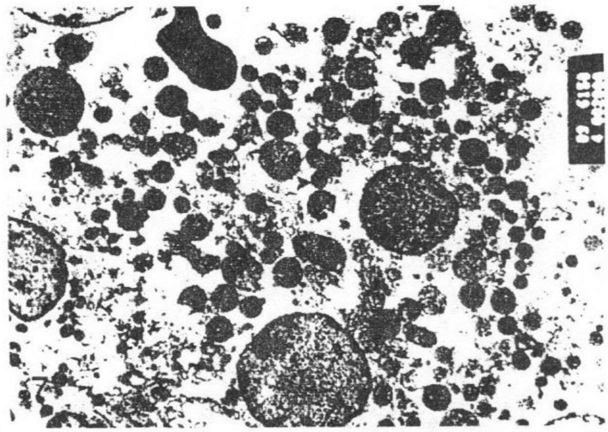


Fig (7) : An electron micrograph of a section in the zona fasciculata of the suprarenal cortex of a stressed rat showing many lysosomes (arrow heads) and dilated sER profiles (tailed arrows). Notice marked lipid depletion (compare versus fig 2). (TEM x 2,800)

Fig (8) : An electron micrograph of a zona fasciculata cell in the suprarenal cortex of a stressed rat showing marked lipid depletion. The mitochondria show loosely packed vesicular cristae with less dense matrix (arrows). Some intact mitochondria (M) can be seen. Notice the dilated smooth endoplasmic reticulum (crossed arrows); some dilated sER profiles contain fluctuant material of low electron density. A myelin figure (arrow head) is observed in the cytoplasm. (TEM x 8,000)

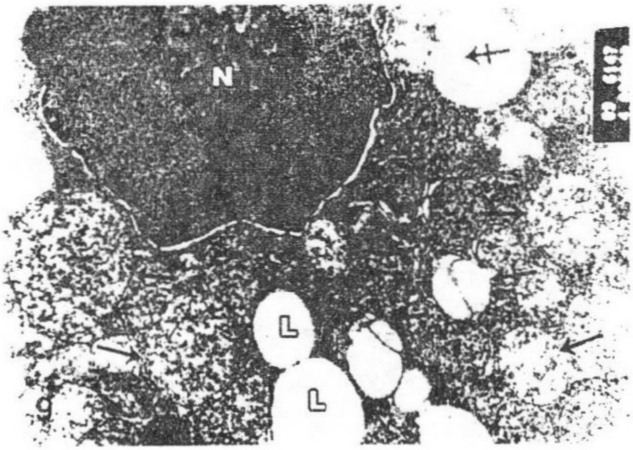


Fig (9) : An electron micrograph of a zona fasciculata cell in the suprarenal cortex of a stressed rat showing a nucleus (N) with irregular border and more condensed chromatin. Large mitochondria with loosely packed vesicular cristae are observed (arrows). Some intact mitochondria (arrow heads) are seen. Few lipid droplets (L) are seen. Notice, the presence of shelf- like protrusion of the inner mitochondrial membrane on the side facing the lipid droplet (crossed arrows). (TEM x 8,000).

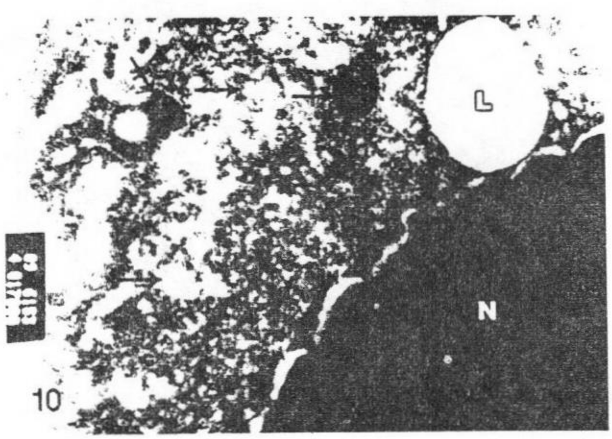


Fig (10) : An electron micrograph of a zona reticularis cell in the suprarenal cortex of a stressed rat showing a shrunken hyperchromatic nucleus (N) with irregular border. The cytoplasm appears non-homogenous. Degenerated ill-defined mitochondria (arrows) are seen. Some intact mitochondria (crossed arrows) are found. Lipid droplets (L) are very few. (TEM x 17,000)

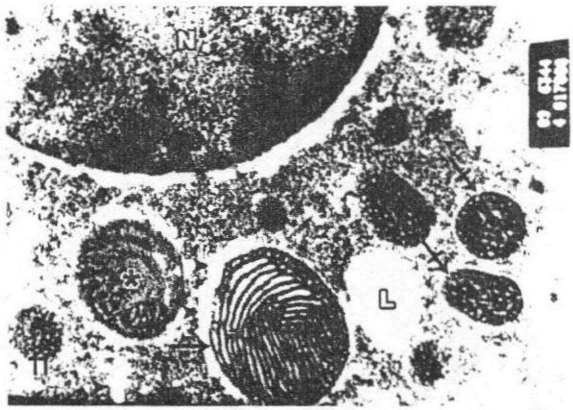


Fig (11) : An electron micrograph of a zona reticularis cell in the suprarenal cortex of a stressed rat showing some intact mitochondria (crossed arrows). A large mitochondrion with parallel-arranged tubular cristae (arrow) and a large degenerating one (star) are seen. Lipid droplets (L) are very few. Note, the dilated smooth endoplasmic reticulum (arrow heads).
(TEM x 17,000)

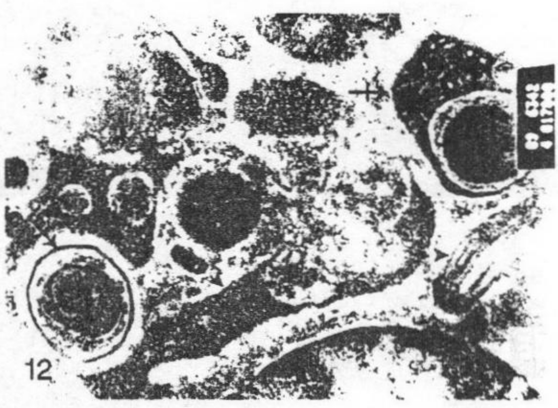


Fig (12) : An electron micrograph of a zona reticularis cell in the suprarenal cortex of a stressed rat showing Bizarre-shaped mitochondria (arrow heads), autophagic vacuoles containing degenerated mitochondria (crossed arrow) all are seen. (TEM x 17,000)

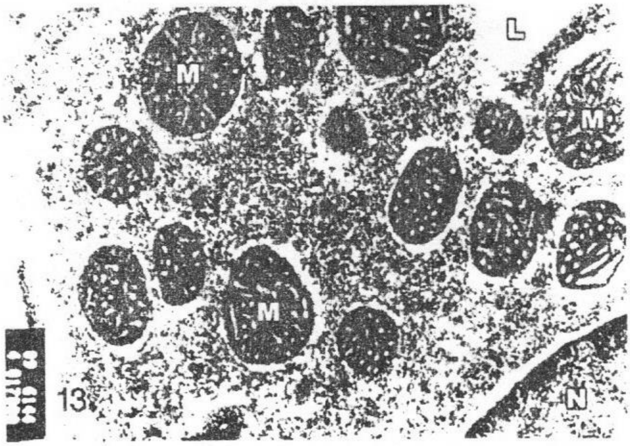


Fig (13) : An electron micrograph of a zona glomerulosa cell in the suprarenal cortex of a stressed rat pretreated with diazepam showing lipid droplets (L) and normal intact elongated mitochondria with lamellar cristae (M), vesicular nucleus (N) also can be detected. (Compare versus fig. 1). (TEM x 17,000)

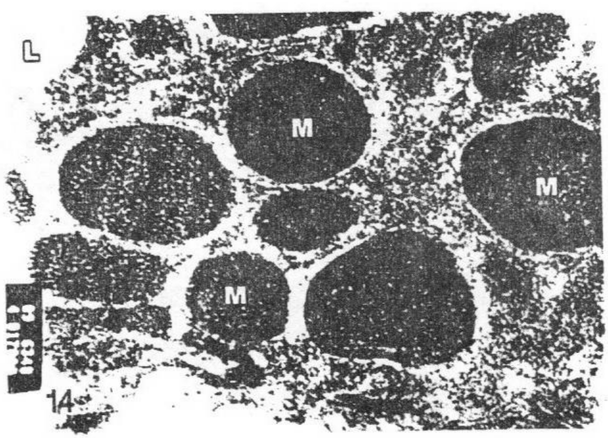


Fig (14) : An electron micrograph of a zona fasciculata cell in the suprarenal cortex of a stressed rat pretreated with diazepam showing lipid droplets (L) and intact mitochondria (M) with densely packed vesicular cristae. (Compare versus fig. 3). (TEM x 17,000)

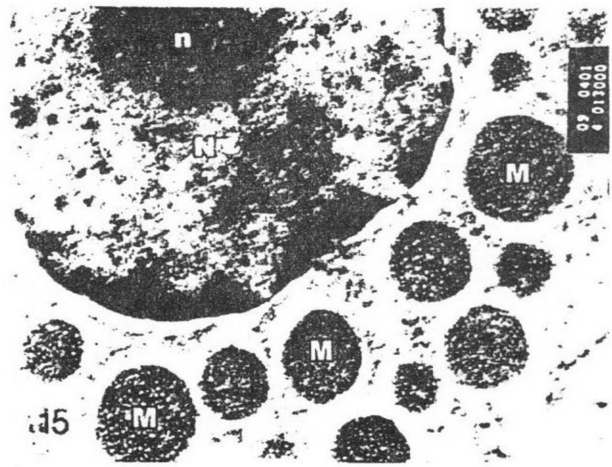


Fig (15) : An electron micrograph of a zona reticularis cell in the suprarenal cortex of a stressed rat pretreated with diazepam showing a vesicular nucleus (N) with prominent nucleolus (n). Mitochondria (M) are spherical with tubulo-vesicular cristae. (Compare versus fig. 4). (TEM x 17.000)

DISCUSSION

Noise represents a physical agent increasingly present in the environment; exposure to noise occurs daily in many environmental conditions which affecting human-being. Loud noise is a very stressful condition leading to ultra-structural changes in several organs like neuro-endocrine glands. Therefore studying the alterations induced by noise could be potentially relevant for environmental medicine (Pellegrini et al., 1997).

Electron microscopic examination of the adrenal cortex of the control rat revealed a structure similar to that reported by many investigators (Fawcett & Jensh, 2002; Young & Heath, 2004; Junqueira & Carneiro, 2005; and Ross & Pawlina, 2006).

In the present study, the serum level of corticosteroid in control rats was 160±7.6 ng\ml. After noise stress, its level was significantly increased, while in stressed rats pretreated with diazepam it was nearly about the normal level. This increase in the serum corticosteroid was explained by Dallman et al. (1992) who stated that there is a major neuro-endocrine mechanism in the stress, which is the activation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in production of corticotropin releasing hormone (CRH) from the paraventricular nuclei of the hypothalamus. CRH will cause rapid increase in the circulating adrenocorticotrophic hormone (ACTH) from the anterior pituitary which in turn stimulates the adrenal cortex leading to subsequent release of corticosteroid (cortisol in animals). Thus, plasma levels of both ACTH and glucocorticoids are a good indicator of stress response, particularly in its acute phase (Pignatelli et al., 1998).

As regard the increase in the serum cortisol level after exposure to noise stress. A similar result was observed by many investigators (Gavrilovic & Dronjak, 2005; Burrow and Campeau, 2005; Alfarez et al .,2006 and Ariizumi & Zheng, 2007). However, the increase in the corticosterone serum level is most likely to be associated with hyper-function of the adrenal cortex, this is confirmed by the ultrastructural modifications that observed in the studied glands. In addition, Nussdorfer (1986) reported that the most involved sub-cellular organelles of zona fasciculata and zona reticularis are smooth endoplasmic reticulum and mitochondria, that play a pivotal role in hormone synthesis and release.

Boyed et al. (1983) stated that the number of lipid droplets in the steroidogenic cells is the expression of their utilization in steroid synthesis as well as the uptake of exogenous cholesterol from the blood stream, as in normal rats the endogenous synthesis of cholesterol from acetate is very low. Only exogenous cholesterol is stored in the lipid droplets. Stress and ACTH stimulate utilization of lipid droplets and increase the number of the receptor sites for LDL and also the activation of endogenous cholesterol synthesis.

Electron microscopic examination of the stressed adrenal cortex in the present work revealed marked changes in some cells of the cortical zones especially in the zona fasciculata and zona reticularis. The zona glomerulosa cells showed no morphological changes apart from the appearance of some rounded mitochondria containing mixture of lamellar and vesicular cristae instead of elongated mitochondria with lamellar cristae. The zona fasciculata cells showed marked changes mainly at the mitochondrial level. Some mitochondria showed cristolysis and contained loosely packed vesicular cristae. Dilated smooth endoplasmic reticulum tubules and lipid depletion were also detected. Protrusion of inner mitochondrial membrane at the side facing the lipid droplet was markedly observed. Zona reticularis showed changes similar to those observed in the zona fasciculata. Moreover, mitochondrial membrane ruptured with non-homogenous cytoplasm, mitochondria with whorled or parallel tubulo-vesicular cristae were observed with giant polymorphic profiles. The nucleus of some zona reticularis cells appeared shrunken with condensed chromatin.

The result of the present work as regard the change of the zona glomerulosa is in agreement with Mazzocchi et al. (1986) who mentioned that after long-term administration of ACTH to rats, there is a significant increase in the volume of the mitochondria and smooth endoplasmic reticulum tubules of the zona glomerulosa cells with transformation of the lamellar cristae of the mitochondria into vesicular ones like those of zona fasciculata cells. They concluded that vesiculation of the lamellar cristae is under the effect of ACTH that not only stimulates the growth of the zona glomerulosa but also transforms its parenchymal cells into cells typical to those of zona fasciculata. Moreover, Soldani et al. (1999) and Gesi et al. (2001) hypothesized that this vesiculation of the lamellar cristae may induce a change in the secretory pattern of the zona glomerulosa that may partly account for the increase in the corticosteroid levels.

Pellegrini et al. (1997, 1998); Soldani et al. (1999) and Gesi et al. (2001) described some mitochondria with diluted matrix and cristolysis in zona fasciculata and zona reticularis after noise stress, other mitochondria with discontinuity of the membranes with consequent communication between the mitochondrial matrix and the cytoplasm were observed. The cells exhibited also a very dense hyaloplasm in which smooth endoplasmic reticulum is dilated in parallel arrays surrounding the outer mitochondrial membranes. The nucleus of some cells were polymorphic with condensed chromatin. Mitochondria with whorled or parallel tubulo-vesicular cristae were also observed together with giant polymorphic mitochondria.

The present study revealed the presence of inner mitochondrial membrane protrusions. Similar finding was seen by Merry (1975) who observed membranous spiral bodies extending from the mitochondrial outer membranes, on the side nearest to the lipid droplets, and penetrated them. The investigator suggested that these protrusions might have some significance in the transport of cholesterol from the lipid droplet to the inner mitochondrial membrane 'desmolase complex', thus facilitating side-chain cleavage of cholesterol to pregnenolone.

Benzodiazepines including diazepam bind with high affinity to specific receptors within the CNS. These benzodiazepines receptors are closely associated with the receptors of gamma amino butyric acid (GABA) which is the major inhibitory neurotransmitter in the mammalian brain. Benzodiazepines binding increases the affinity of the GABA receptor for GABA hence benzodiazepines potentiate GABA-ergic neurotransmission in all areas of the CNS (Mikkelsen et al., 2005).

Regarding the decrease in the cortisol level in diazepam pretreated rats as compared with that of stressed animal, similar results recorded by many investigators, who had studied the relation between plasma corticosteroid level and diazepam administration in stress conditions. Diazepam not only prevents the increase in corticosteroid level but also decreases the corticosteroid production rate (Erdosova et al., 1977 and Le Fur et al., 1979).

In this study, ultrastructural examination of the adrenal cortex of stressed rat pretreated with diazepam revealed that diazepam reduced the ultrastructural changes induced by stress. Similar data were observed by many investigators (Pellegrini et al., 1998 and Beszczynska & Siejka, 2003).

The results of the present study proved that diazepam reduces the ultra-structural changes induced by noise stress in rat's adrenal cortex, most probably this occurs at a central level. Therefore, administration of diazepam in stress condition is recommended to protect the adrenal gland from these changes induced by elevated plasma ACTH.

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الملخص العربى

تأثير الديازيبام على لحاء الكظرى للجرذ الأبرص المُعرض لإجهادِ الضوضاءِ. دراسة بالمجهر الإلكترونى

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إستهدَفتْ هذه الدراسةِ إستعَرَاض تأثيرِ تعاطى الديازيبام على التعديلاتَ التركيبية الدقيقة المُحدثة بالضوضاءِ فى لحاءِ الكظري للجرذِ.

إستُعملتْ فى هذه الدراسةِ الجرذان البرصاء البالغة لكلا الجنسين. و قد قُسمتْ الحيوانات إلى ٣ مجموعاتٍ؛ المجموعة الضابطة و المجموعة المُعرَضَة للضوضاء (٦ ساعاتٍ يومياً لمدة ١٤ يومِ) و المجموعة المحَقونة بالديازيبام فى التجويف البريتونىَ (نصف ملليجرام لكل ١٠٠ جرام من وزن الجسمِ، ٣٠ دقيقة قبل التعرّضِ للضوضاء). تم قياس و عمل دراسه كمية إحصائية لكمية الكورتيزول فى المصل بالإضافة إلى دراسةِ بالمجهرالإلكترونىِ لقطاعات بالغة الرقة للغُدد الكظريةَ.

فى المجموعة الضابطةِ، وُجِدَ أن مُستوى الكورتيزول فى المصلَ كانَ ١٦٠ ± ٧,٦ نانوجرام/ مليلتر. كَشفَ فحصُ المجهرِ الإلكترونىِ لخلايا الطبقة العقدية وجود ميتوكوندريا مُطَوَّلة بها أرفُف على هيئة صفائح رقيقة. إستعَرضتْ خلايا المنطقة الحزمية كمية كبيرة مِنْ قطراتِ الدهون ووفرة من الشبكة الإندوبلازمية الملساء. كَانتْ الميتوكوندريا كُروية الشكلِ مَع أرفُف على هيئة حويصلات مكتظة بشكل كثيف. أما بالنسبة لخلايا المنطقة الشبكية، فقد أظهرت قطرات دهنية أقل و ميتوكوندريا أكثر والتى كَانتْ مُدوَّرة أو كُروية مَع أرفف انبوبية-حُويصلية مكتظة بشكل كثيف.

بعد التعرض لإجهادِ الضوضاءِ، إزدادَ مُستوى الكورتيزول فى المصلَ بشكل ملحوظ. و لم تُظهرْ

خلايا الطبقة العقدية أى تغييرات تركيبيةِ ما عَدا ظُهور بعض الميتوكوندريا المُدورة المُحتوية على خَليْطَ من الأرفف الصفائحية و الحويصلية. وقد أظهرت الميتوكوندريا فى بعض خلايا الطبقتين الحزمية و الشبكية تحلل فى الأرفف و تم إكتشاف بَعْض الميتوكوندريا العملاقة, كما كان هناك إتّساعَ فى الشبكة الإندوبلازمية الناعمة. كَشفتْ خلايا الطبقة الشبكية وجود بَعْض الميتوكوندريا ذوات شُكّل غريب مع تمزيق غشاء الميتوكوندريا. وقد بَدتْ بَعْض النُوى مُنكمشةً مَع إحتوائها على كروماتين مكَثّف.

وعلى الجانب الآخر, فقد لُوحظ إنخفاض ملموس فى مُستوى الكورتيزول إلى حوالى المُستوى الطبيعي فى مصل الجرذان المرهقةِ بالضوضاء والمُعَالَجة من قِبْلَ بالديازيبام. ولم تُرَىْ أى تغييراتَ تركيبية دقيقة قابلة للكشف فى لحاء الكظري.

من المُمْكِنُ أَنْ يُستَنتجَ بأنَ، وظيفة وتركيب لحاء الكظري قد تأثّرا بالتعرّض لإجهاد الضوضاء. هذه التاثيرات قد إنخفضَت لدرجة كبيرة بتعاطى الديازيبام قَبْلَ التعرض للضوضاء. لذا، يُوصى بتعاطى الديازيبام فى حالات الإجهاد لكى يَحْمى الغُدّة الكظريةَ مِنْ تأثيرِ الإفراز المُتزايد للهرمون المُحفز للحاء الكظري.