Histological and Histomorphometric Reorganization of the Adrenal Cortex of Adolescent Male Albino Rat Following Exposure to Stress


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Abstract

Background: Stress results in histological as well as morphometric changes in the adrenal cortex. This study is to demonstrate these changes during adolescent stage of development in the male albino rat.

Material and Methods: Eighty male adolescent albino rats (4-6 months old) of Wistar strain were included; they were divided into two groups; the control group and the stressed group. The stressed group was subjected to restraint stress for 14 days. The glands were subjected to light microscopic examination using Hx & E and electron microscopic examination using transmission electron microscope. Quantitative histomorphometric estimations included morphometric measurement for thickness of the three adrenocortical zones.

Results: Imposing stress resulted in changes in the histological features of the adrenal cortex as well as changes in the size of the different adrenocortical zones, the most remarkable of which was increase in the thickness of the ZF and ZR and decrease in the thickness of the ZG. This difference was found to be statistically significant. Ultrastructural changes included the appearance of indented nuclei and occasional shrunken ones as well as dense lipid droplets.

Conclusion: That adrenal cortex becomes modified in its cytological characteristics and proliferative features in response to stress. This further highlights the relevance of exploring and understanding the contribution of these adrenocortical changes to many of stress-related diseases.

Key Words: Stress – Adrenal Cortex – Adolescence – EM.

Introduction

STRESS is a state of disturbed homeostasis due to internal or external sources such as physical or psychological stimuli known as stressors. Stress responses are mainly mediated by the activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis [1]. Thus, the adrenal gland is considered the primary peripheral endocrine gland in the stress response [2].

Extensive interest has been given to restraint as a model of stress induction. Researches have focused on implications of the restraint model in determining the stress-induced responses on various aspects of the HPA axis [3,4].

Stress-induced HPA axis reactivity is higher during adolescence compared to neonatal or adult stages of development and that substantial changes in stress reactivity and hormonal response to stress have been noted to occur also during this time [5].

Aim of work:

The aim of the present work was to demonstrate the histological, as well as the morphometric changes occurring in the adrenal cortex following exposure to restraint stress in the adolescent male albino rats.

Material and Methods

This study was carried out on 80 male adolescent albino rats (4-6 months old) of Wistar strain obtained from the animal house of Kasr El-Ainy, Faculty of Medicine, Cairo University. This work started on 2014 and ended on 2016.

Rats were divided into two groups; a control group and a stressed group.

The experiment lasted 14 days. All control rats were housed individually in standard stainless steel cages with galvanized iron wires. The size of the control cages were 41 X 28 X 19cm to allow the animals to move freely.

Restraint stress was performed for 2 hours daily throughout the 14 days of the experiment by placing...
each animal in a plastic bottle in such a way that only minimum mobility is possible. The size of the plastic restraints was 6cm diameter X 20cm long [6].

The study was performed in the animal house of Kasr El-Ainy, Faculty of Medicine, Cairo University. All rats were housed under standard laboratory conditions at 22-24ºc with 12 hour light/dark cycle and were fed on a constant adequate nutrition and allowed free access to drinking water ad libitum.

The experiment was conducted in accordance to the guidelines of the committee of laboratory animals at Kasr El-Ainy.

Stress protocol:

All stress animal groups were exposed to these restraint conditions for 2 hours daily at random times during the light period of the light/dark cycle to avoid habituation during the 14 days of the experimental procedure [7]. On the specified day, the animals were sacrificed under non stress conditions by rapid cervical decapitation, their abdomen were opened through a ventral midline incision, peri-adrenal fat was removed and the adrenal glands of both sides were rapidly dissected out.

The left adrenal glands were used in the light microscopic study while those of the right side were used in the electron microscopic study.

The Adrenal glands were subjected to the following:

A- Light microscopic examination: Paraffin technique [8]:

The left adrenal glands from all groups were dissected, fixed in 10% buffered formaline overnight at room temperature, dehydrated in alcohol, cleared in xylol and paraffin blocks were prepared. Sections of 5µm thickness were cut and stained with Hematoxylin and Eosin (Hx & E) stain [9].

B- Electron microscopic examination [9]:

The suprarenal gland on the right side was prepared for ultrastructural examination using Transmission Electron Microscope (TEM).

C- Quantitative morphometric study:

Quantitative morphometric estimation was done using image analyzer (Leica Imaging System Ltd., Cambridge, England) (Fig. 1). Images were captured live on to the screen from sections under a light microscope (Olympus Bx-40, Olympus Optical Co. Ltd., Japan) with an affixed video camera (Panasonic color CCTV camera, Matsushita Communication Industrial Co. Ltd., Japan). The video images were digitalized using “Leica Qwin 500C” which is a Leica’s windows based image analysis tool kit. The quantitative morphometric study included morphometric study for thickness of adrenocortical zones where the mean thickness of the three classical zones of the adrenal cortex was measured in ten serial Hx & E stained sections in each animal. The obtained data were tabulated and subjected to statistical analysis.

Statistical analysis:

The obtained data were analyzed using SPSS (statistical package for social sciences) version 15. The numerical data were described as mean ± Standard Deviation (SD). The statistical significance of the difference between all the obtained mean values was assessed using Student’s “t” test.

The probability level (p-value) ≤0.05 was considered as significant. p-value ≤0.01 was considered as highly significant.

Results

Control group:

1- Light microscopic results:

Examination of haematoxylin and eosin stained sections of the adrenal cortex of the adolescent control albino rats showed a capsule beneath which the three layers of the adrenal cortex exhibited their characteristic arrangement. The ZG cells were arranged in arches, ZF cells in parallel longitudinal cords one or two cell thick while the innermost darkly stained ZR was disposed in the form of anastomosing network of cells (Fig. 2).
2- Electron microscopic results:

Ultrastructural examination of the ZG of this group showed some cells with prominent nucleoli and others displaying nuclei with euchromatin and peripheral heterochromatin with their cytoplasm crowded with a huge number of lipid droplets (Fig. 3).

The ZF cells appeared polygonal with indented nuclei and cytoplasm heavily loaded with lipid droplets. Endothelial lined blood capillaries were also observed (Fig. 4).

The ZR cells exhibited lipid droplets as well as numerous mitochondria with closely packed tubular cristae (Fig. 5).

Stressed group:

1- Light microscopic results:

Examination of haematoxylin and eosin stained sections of the adrenal cortex of the adolescent stressed albino rats revealed the appearance of the so called Zona Intermedia (ZI) which is a narrow zone of closely packed cells situated between groups of ZG cells and cords of ZF cells (Figs. 6, 7).

Also, groups of dark cells with highly acidophilic cytoplasm were illustrated in the ZF (Fig. 7) as well as in the juxtamedullary portion of the ZR (Fig. 8).

2- Electron microscopic results:

ZG cells appeared polyhedral with indented nuclei and occasional shrunken ones. Their cytoplasm contained numerous lipid droplets (Fig. 9).

The cytoplasm of ZF cells had numerous mitochondria some of which contained elongated or spherical electron dense clumps. Lipid droplets in this zone appeared packed and acquired an irregular outline. Secondary lysosome and scattered free ribosomes were also noticed (Fig. 10).

ZR cells appeared with irregular nuclei, with disturbed nuclear contour and clumping of their heterochromatin. The cytoplasm was studded with mitochondria some of which showed disrupted cristae. Vesicles of Smooth Endoplasmic Reticulum (SER) were distributed throughout the cytoplasm. Dense lipid droplets as well as occasional light lipid droplets were also seen (Fig. 11).
Fig. (5): An electron micrograph of ZR of adolescent control group showing numerous mitochondria (M) with closely packed tubular cristae. Lipid droplets (L) are also seen. (TEM x15000).

Fig. (6): A micrograph of a section in the adrenal cortex of the adolescent stressed group showing groups of ZG cells and cords of ZF cells with a narrow zone of closely packed cells (circle) of zona intermedia (ZI) inbetween. (Hx & E x200).

Fig. (7): A micrograph of a section in the adrenal cortex of the adolescent stressed group showing dark cells with highly acidophilic cytoplasm present in the ZF arranged in the form of parallel cords (arrows) or exhibiting patchy distribution (black circles). The ZI (white circle) is also found intervening between the ZG and ZF. (Hx & E x200).

Fig. (8): A micrograph of a section in the ZR and adjoining adrenal medulla (M) of the adolescent stressed group showing layers of dark cells (arrow) in the juxtamedullary portion of the ZR. (Hx & E x200).

Fig. (9): An electron micrograph of the ZG of the adolescent stressed group showing polygonal ZG cells exhibiting a nucleus (N) with irregular outline and nuclei with indentation of their nuclear envelope (white arrows). Multiple lipid droplets (L) are also observed. (TEM x8000).

Fig. (10): An electron micrograph of ZF of the stressed group showing multiple mitochondria (M) of variable sizes, some of them contain elongated or spherical electron dense clumps (white arrows). Numerous packed lipid droplets (L) with irregular outline, secondary lysosome (long black arrow) and scattered free ribosomes (short black arrow) are also noticed. (TEM x10000).
Fig. (11): An electron micrograph of ZR of the stressed group showing an irregular nucleus (N) with a disturbed nuclear contour (white arrow) and clumping of its heterochromatin (white arrowhead). The cytoplasm is studded with mitochondria (M) some of them show disrupted cristae (black arrows). Vesicles of SER (S), dense lipid droplets (L) as well as occasional light lipid droplets (asterisk) are also seen. (TEM x15000).

- **Morphometric study for thickness of adrenocortical zones:**

  Measurement of the thickness of the adrenocortical zones showed that imposing stress to the adolescent rats resulted in decrease in the mean thickness of the ZG and increase in the thickness of the ZF and ZR (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Thickness (µm) (Mean ± SD)</th>
<th>ZG</th>
<th>ZF</th>
<th>ZR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent control</td>
<td>44.7±4.5</td>
<td>159.3±8.3</td>
<td>95.7±5.8</td>
<td></td>
</tr>
<tr>
<td>Adolescent stressed</td>
<td>31.5±7.2</td>
<td>179.7±5.7</td>
<td>112.3±2.5</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>8.2</td>
<td>4.7</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Degree of increment/ decrement</td>
<td>29.5%</td>
<td>12.8%</td>
<td>17.3%</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.01**</td>
<td>0.043*</td>
<td>0.041*</td>
<td></td>
</tr>
</tbody>
</table>

* : Significant (p<0.05).
** : Highly Significant (p<0.01)

**Discussion**

A striking stress-induced finding in the present study was the appearance of a discrete layer of proliferating cells at the junctional zone between ZG and ZF in the adolescent stressed rats. This layer was about three to four cells in thickness. The histological features of this zone are similar to those of the formerly named “the zona intermedia” [10] “the transitional zone” [11], or “the undifferentiated zone (ZU)” [12].

This zone is devoid of any significant endocrine functions specific to any of the three classical adrenocortical zones, therefore, the author designated this zone as “undifferentiated cell zone (ZU)” [12]. Cells in this zone could proliferate and migrate bidirectionally; toward the ZG centrifugally and toward the ZF centripetally thus elucidating this zone as a stem/progenitor cell zone that is a renewal source of cells, capable of differentiating into zone-specific cells. Cells differentiating to the ZF migrate inwardly and then transform into the ZR cells. They finally degenerate at the border between the cortex and the medulla [12].

The adrenal cortex of the adolescent stressed group in the present study exhibited the presence of dark cells with highly acidophilic cytoplasm in the ZF. These cells were arranged in the form of parallel cords or exhibiting patchy distribution. Similar observations were made by [13] who reported that the stressed adrenal glands were characterized by the presence of plump of cells with greatly increased eosinophilia and density of the cytoplasm instead of the usual pale staining clear ZF cells with somewhat foamy cytoplasm. These changes correlate well with adrenal stimulation.

These layers of dark cells were also encountered in the juxtamedullary portion of the ZR in the present study matching to the observations reported by [14] who found variable numbers of dark cells with shrunken nuclei and denser cytoplasm near the medulla.

The ultrastructural remodeling of the adrenals of animals of the stressed groups in the present work was mainly manifested as changes in the constituents involved in steroid hormone biosynthesis, namely, the lipid droplets, mitochondria and SER.

**Conclusion:**

The adrenal cortex displayed observable histological and morphometric changes in response to stress during the adolescent stage of development. In this regard, chronic stress has been proposed as one of the key factors predisposing to endocrine diseases.

**References**

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