

Original article:

Histological changes induced by stress in Adrenal gland of albino rats

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Abstract:

Introduction: The separation of Psychology from the premises of Biology is purely artificial, as the human psyche lives in dissoluble union with the body. Response to stress involves multiple hormones such as ADH, ANGIOTENSIN, GLUCAGON AND GROWTH HORMONE. Rivier and Plotzky (1986) stated that CRH-ACTH - CORTISOL AXIS is central to these integrated responses to stress. Psychological changes can disturb the function of any organ and bring about somatic manifestations, the neuroendocrine effects of stress are mediated by hypothalamus (Mayfield 1980) as it serves as a transducer that translates higher neural activity into physiological responses, as on adrenal gland, release of corticotropin releasing factor (CRF) in response to stress from the hypothalamus causes release of ACTH from pituitary, which acts on adrenal cortex and medulla to produce CORTISOL AND CATECHOLAMINES to deal with stressful events overall effects of these changes is to prepare the body for FIGHT / FLIGHT.

If this stress is chronic, significant histological changes are observed in adrenal cortex and medulla.

Material and methods: An observational study was done in sixty albino rats, divided into 3 groups of 20 each with ten males and ten females in each group, Histology of adrenal gland was studied after processing and staining with H & E Stain, Giemsa stain in immobilized rats for different durations, (IMMOBILIZATION STRESS WAS CHOSEN AS IT IS NOT PHYSICALLY PAINFUL TO RATS AND SERVED AS BOTH PHYSICAL AND PSYCHOLOGICAL STRESSOR) maximum upto 3 months as control, test and diazepam treated groups, animals were sacrificed adrenal glands were identified and taken out, preserved and studied for histological changes.

Observations: study revealed increased cortical thickness which was found maximum at the end of three months, multiple ischaemic areas were observed in cortex, intracytoplasmic granules were seen in both. Cortex and medulla, all in favour of cellular hypertrophy and hyperplasia, all the changes were more pronounced in stress group males, diazepam treated group showed less pronounced effects.

Result: Significant increase found in escape behavior and aggressiveness of the animal, histological changes were in favour of hypertrophy and hyperplasia in cortical region, medulla did not show much change except. Increased number of intracytoplasmic granules, diazepam partially antagonized the effect of stress on all the parameters observed.

Key words: neuroendocrine, stress, hypertrophy, hyperplasia

INTRODUCTION

It has been proved through countless studies that our mental attitude has powerful influence on our physical health. The body and the mind are not two separate entities – all kinds of mental or physical stressors simultaneously influence the body and the mind and leads to psychosomatic disease. Mental stimuli and emotions can cause bodily change as effectively as bacteria and toxins. The psychological change may disturb the function of any organ of the body with somatic manifestation. In the present study it was intended to study the effect of immobilization stress which acts as physical as well as psychological stressor, on the histomorphology of adrenal gland. Effect of stress over adrenal gland is mediated by hypothalamo-pituitary-adrenal axis (CRH-ACTH-CORTISOL AXIS)

HYPOTHALAMUS: has been called the HEAD GANGLION OF AUTONOMIC NERVOUS SYSTEM as the autonomic responses triggered in hypothalamus are part of complex phenomenon such as rage and emotions which occur as a result to any sort of stress on human body , Actually the neural basis of instinctual behaviour and emotions lie es in the limbic system . Hypothalamus and limbic system are intimately connected to each other and are concerned with feeding sexual behaviour , emotions rage fear and motivation , Hypothamus secretes corticotropin releasing hormone which in turn acts over anterior pitutary to cause secretion of ACTH which act over adrenal cortex and medulla to produce increased cortisol and adrenaline .

Study of Guilleman and schalay (1978) and Wade (1978) identified many peptides that serve as hypothalamic releasing and inhibiting factors , some of these play neuroendocrine effects as Endorphins and Enkephlins .

Evans et al (1986) reported that opiate receptors have been found through out the CNS and in. Some peripheral sites such as adrenal and lymphocytes . B Endorphin release is increased by stress and. General role of endogenous opioids include regulation of pain anxiety and memory along with buffering the adverse consequences of stress .

MATERIAL & METHODS

The present study was carried out on sixty albino rats divided into three groups of twenty each with ten males and ten females in each group. Further subgrouping was done and each group was divided into five group of four rats, each having two males and two females in each group. The three groups of rats were labelled as GROOUP A , GROUP B. AND GROUP C

The rats of group A were taken as control. They were housed in large wire cage and were free to move.

The rats of group B were immobilized in small transparent plastic jars with five holes, for four hours, daily, for 3 months the rats of group B were immobilized in similar manner and were given injections of diazepam 0.15mg/100gm of body weight during the period of stress Diazepam was used to eliminate the stress. All the animals were sacrificed over the time span of three months altogether ranging from first ,third , seventh and fifteenth day of starting experiment and at the end of three months adrenal glands were identified and dissected out .

Gross exm: Adrenals were found as dark red rounded structures situated on superior border of kidneys on either side of vertebral column .

After thorough washing of tissues with normal saline, tissues were kept in preservatives 10% formaldehyde 3% potassium dichromate ,then processed for paraffin section and stained with Hematoxylin eosin and Giemsa stains.

Observations of the slides were done under OLYMPUS TRINOCULAR RESEARCH MICROSCOPE.

OBSERVATION

CONTROL GROUP :

Male : There was evidence of a thick connective tissue capsule form which connective tissue trabeculae were seen passing into the cortex of gland. Adrenal cortex was divided into three concentric zones not sharply demarcated from each other. Outer zone was **Zona-Glomerulosa** in which cell arrangement was in provide droplets. Vacoules were seen in the cytoplasm. The nuclei of cells were stained in violet colour. Middle layer was zona fasciculata in which cells were arranged into cords. Cytoplasm was some what granular and vacuolar. It was pale in appearance. The cells bordering the adrenal medulla were zona reticularis. Medulla showed rounded cells with granules, blood sinuses and symphatetic ganglion.

Female : The appearance of the tissue was more or less similar to the male. The histological appearance of the adrenal gland in the control group in all the sub groups (based on the day of sacrifice and both sexes was almost similar.)

STRESS X 1 DAY

(Rates were sacrificed after 5 hrs of immobilization stress).

STRESS GROUP :

Male : There was evidence of a thick connective tissue capsule along with indentation of capsule in the substance of cortex in form of trabeculae.

Zona glomerulosa : It showed rounded cells with vacuoles inside. Nuclei were stained in violet colour.

Zona fasciculata : It showed cords of cells with spaces between the cords along with granulated cytoplasm. Cytoplasm was pale in colour. Vacuoles were present in the cytoplasm.

Zona Reticularis : It showed network of cells.

Medulla : There was evidence of rounded cells with granules in the cytoplasm, blood sinuses and sympathetic ganglion.

Female : Thick connective tissue capsule and trabeculae were found as such.

Zona glomerulosa : Its appearance was almost similar to the male.

Zona fasciculata : It showed cords of cells with spaces between the cords along with pale granulated Vacuoles were present in cytoplasm.

Zone reticularis : It showed network of cells.

Medulla: It was similar to that of male .

Stress with diazepam group:

Male : No histological change was observed from the control rats,

Female : almost similar to control female

There was almost no changes in the histological appearance of the adrenal gland in both sexes, stress and stress with diazepam group on the first day of immobilization stress .

STRESS X 3DAYS

(Rate were sacrificed on the 3rd day of giving immobilization stress)

STRESS GROUP:

Male: Cortical thickness was apparently increased. Connective tissue capsule and trabeculae were found as such.

Zona glomerulosa: Here the thickness of the layer was increased. More layer of cells of round/ovoid shape were seen.

Zona fasciculata : Cells were arranged in form of cords. However there were 2-3 patchy areas of cell compaction.

Cytoplasm was much more granulated than the cytoplasm of fasciculata cells of control rats. Cytoplasm was pale in colour except some small areas of eosinophilic cytoplasm. Vacuoles were seen in the cytoplasm.

Zona Reticularis : Network of cells were present. However this layer was also thickened apparently.

Medulla : Rounded cells were seen which had multiple granules. Blood sinuses were also seen in between. Sympathetic ganglion were also present.

Female : Cortical thickness was apparently increased. Connective tissue capsule and trabeculae were found as such.

Zona glomerulosa: Thickness of this layer was increased.

Zona Fasciculata : Histological appearance of this layer was more or less similar with the appearance of the fasciculate cells of control female rat except some areas of patchy compaction of cells.

Zona Reticularis : Widening of reticularis was seen in comparison of control female rats.

Medulla : Rounded cells with granules were present with blood sinuses in between.

STRESS WITH DIAZEPAM GROUP:

Male : Histological appearance of the adrenal cortex and medulla was same like of stress male group.

Female : Histological appearance was similar to stress female group. There were stress changes in the adrenal cortex in form of-

1. Increased cortical thickness.
2. Patchy areas of cell compaction.
3. Patchy areas of eosinophilic cytoplasm.
4. Widening of reticular layer.
5. More granules in the cytoplasm of medulla and cortex.

Injection of Diazepam did not alleviate stress.

STRESS X7 DAYS

(Rats were sacrificed after giving immobilization stress of 7 days)

STRESS GROUP :

Male: Cortical thickness was much more increased. Zona glomerulosa : Thickness of this layer was increased as well as the number of granules in the cytoplasm.

Zona fasciculata : This layer was also widened along with more areas of cell compaction there for leading to the appearance where some areas showed highly compacted cells and some areas showed much widened spaces between the cells cords. Cytoplasm was more eosinophilic.

Zona Reticularis : This layer was also widened along with increased number of granules inside.

Medulla : There were rounded cells along with multiple granules, blood sinuses and sympathetic ganglion.

Female: Cortical thickness was not that much increased as in the male rats otherwise the histological appearance was similar..

STRESS WITH DIAZEPAM GROUP:

MALE : Histological changes were the same like that of stress group but less pronounced.

Female : Changes in the adrenal gland were almost similar to that of control female rat.

Histological appearance showed that stress changes were more pronounced in males.

Diazepam alleviated the stress.

STRESS X 15 DAYS

(Rats were sacrificed after giving immobilization stress for 15 days)

STRESS GROUP :

Male: Cortical thickness was increased, capsule and trabeculae were found as such. All the three layers of cortex were thickened along with more granules their cytoplasm.

Zona fasciculata : It showed eosinophilic cytoplasm, more areas of cell compaction and increased number of granules in the cytoplasm.

Female : Similar but less pronounced changes were found.

STRESS WITH DIAZEPAM GROUP :

Male : Histological presentation was similar to control female.

Observation showed the stress changes were more pronounced in male rate.

Diazepam alleviated the stress.

STRESS X 3 MONTHES

(Rats were sacrificed after giving immobilization stress for 3 months)

STRESS GROUP:

Male: Cortical thickness was very much increased.

Zona glomerulosa : It was very much widened along with multiple granules in its cytoplasm.

Zona fasciculata : cytoplasm of cells was dark pinkish (eosinophilic). Cells were highly compacted. Patchy ischaemic areas were also visualized.

Zona Reticularis: It was grossly widened.

Medulla : It showed increased number of granules in its cells.

Female : Same histological changes were seen they were less pronounced.

STRESS WITH DIAZEPAM GROUP:

Male: Histological appearance was similar to the control male rats.

Female : Histological appearance similar to control of rats.

Stress changes were more pronounced in male rats.

Diazepam treated group did not show stress changes.

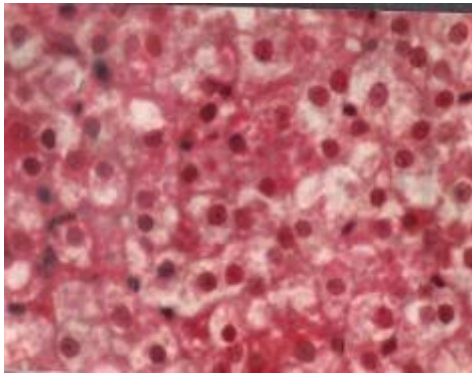
DISCUSSION

There was appearance of increased cortical thickness which was found maximum at the end of three months starting gradually from 3 days. All the three layers of cortex were increased in thickness. Small patchy areas of cell compaction in zona fasciculata were seen in groups sacrificed on seventh, fifteenth day at three months. Cytoplasm of the cells of zona fasciculata became eosinophilic and at the end of three months whole of the cell layer showed eosinophilic cytoplasm. Multiple ischaemic areas were seen in the cortex of gland at the end of three months. Intracytoplasmic granules were seen both in cortex and medulla. Their number was maximum in stress group males. All these changes were more pronounced in stress group males. Stress changes were present in stress group female but they were less pronounced. Diazepam treated group showed almost similar histological changes at the end of three days but 3rd day onwards these changes were reverted back to normal i.e. cortical thickness was not that much increased along with very few areas of cell compaction and eosinophilic cytoplasm in zona fasciculata. Reticular layer thickness was almost normal medulla showed less number of granules.

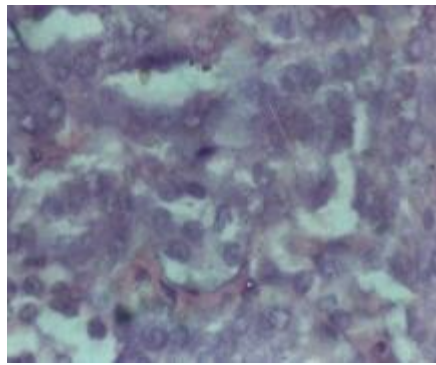
CONCLUSION

Thus the following conclusion were drawn from the above study.

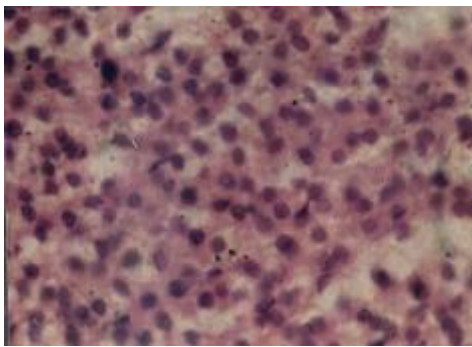
1. Stress caused increase in thickness of adrenal cortex with increased thickness of individual layers also.
2. Cytoplasm of cells of Zona fasciculata became eosinophilic along with more number of granules in both cortex and medulla due to stress.
3. Cells of Zona Fasciculata became more compacted there for the spaces between the cell cords became more widened.
4. Reticular layer became widened.
5. Medulla did not show much changes except increased number of granules in stress group.



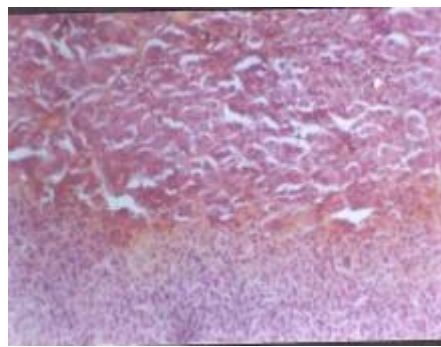
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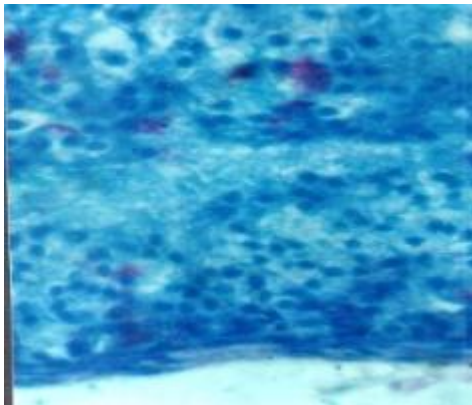
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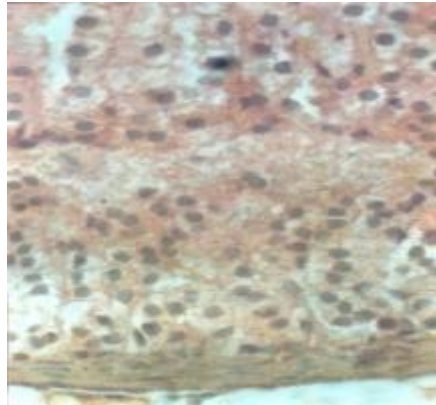
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