A CONTRASTIVE EXAMINATION OF ADRENALECTOMY ON THE
HEPATIC AND REPRODUCTIVE TISSUE OF THE MALE AND
FEMALE ALBINO RATS

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ABSTRACT

A significant decrease in the levels of total proteins along with an increase in the levels of free amino acid (FAA) and ammonia and activities of protease, alanine aminotransferase (AIAAT), aspartate amino transferase (AAT) and glutamate dehydrogenase(GDH) were observed in the penis of male and vagina of female rats on adrenalectomy (ADX) at day 15 and day 30 compared to sham operated (SO) rats. These changes indicated active proteolysis and trans-deamination in the reproductive tissues of rats on adrenalectomy. The magnitude of the changes were significantly more in the vagina of female ADX rats than in the penis of males. Longer the duration of adrenalectomy more the protein breakdown in both the sexes as noticed from day 15 to day 30. Corresponding to the changes in protein levels, some degenerative changes in the histology were observed in the penis and vagina of ADX rats at day 15 day 30 compared to normals. A mild damage was seen in areolar connective tissue and elastic fibres of penis at days 15. On days 30, the three erectile tissues of penis overlapped with irregular arrangement with the congestion of areolar tissue. On 15 days of ADX female rats, the vagina showed less number of leucocytes and wide spaces appeared between elastic fibres of fibrosa. On day 30 the vagina exhibited the presence of very less number of leucocytes in mucous while the elastic fibres of muscularis mostly disappeared. The observations indicated proteolysis and active trans-deamination in the reproductive tissues of rats on adrenalectomy. It could lead to structural disruption and decreased reproductive ability.

KEY WORDS: Albino rats, Adrenalectomy, Histology

INTRODUCTION

The classic endocrine glands are the pituitary, adrenals, thyroid, parathyroids, pancreatic isletes, gonads and placenta. Apart from that adrenal gland is an important endocrine gland which secretes hormones concerned with carbohydrate, proteins and lipid metabolisms, balance of electrolytes in blood, maintenance of circulatory blood volume, control of sexual maturity and regulation of extracellular fluid volume. Adrenal gonadal interaction appears to depend upon overlapping function of the steroid hormones of the adrenal gland and gonads on the reproductive organs and stress operated mechanisms as reported by Goncharov et al. (1984). The process of reproduction is a complicated and intricately synchronized phenomenon. The organs that take part in this mechanism will function perfectly in co-ordination with each other. Any stress on an animal invokes compensatory metabolic adjustments in its organs through modification and modulation of the quality and quantity of various biochemical constituents and enzymes (Assem and Hunke, 1983). Removal of gland
would deprive the organism at various levels if its normal source of hormones are not available. Measurable abnormalities appear in the individual during its life history. Thus, bilateral removal of adrenal gland to a number of metabolic disturbances which are identical with those appearing in patients with Addison’s diseases, such as extreme muscular weakness, a variable degree of hypoglycemia, ceased growth in young animals, loss of body weight, electrolyte imbalance and decreased reproductive function (De GRoot and Jameson, 2001). Adrenal gland is an important role in the maintenance of the penile erection and also which is an androgen-dependent organ (Mills et al., 1996). If any deficiency of adrenal hormone can lead to failure of penile erection in ADX rats (Penson et al., 1997). Vagina is also estrogen dependent organ that undergoes a drastic changes in the cells during estrous cycle in ADX rats (Venkata Reddy et al., 2007). Several reports though are available on the general impact of adrenalectomy but very little information is available on the protein levels and histological changes of reproductive tissues. Therefore, the present work is aimed to understand such changes in the reproductive tissues of male and female adrenalectomized rats in order to correlate them to implication of Addison’s disease.

EXPERIMENTAL DESIGN:
Rats were divided into 3 groups, each group consisted of 12 individuals. Of this, six were males and remaining six were females. First group of rats were called as sham operated (SO) in them the adrenal glands were kept intact and considered as control. The second and third groups of rats were bilaterally adrenalectomized (ADX) by the dorsal approach in a single stage of operation as followed by Russo et al. (2003) and those two group of animals were considered as experimentals; one was maintained for 15 days and the other was for 30 days. The rats were anaesthetized during surgery with ketamine (80mg/kg body weight) plus xylazina (12mg/kg body weight) administered intraperitoneally in a volume of 0.3ml. ADX rats were given 0.9% physiological saline as drinking water to compensate the loss of salts and SO rats were given normal tap water. All rats were housed and cared according to the guide for the care and use of laboratory animals (Mitruka et al., 1976). The ethical committee permission has been obtained by the department to carryout the research work on rats. After the stipulated period, the testis of males and ovary of females were isolated for the estimation of the following parameters of protein metabolism. Histological sections of penis and vagina of both ADX and SO rats were also taken.

The level of total proteins was estimated by Lowry et al (1951). Free amino acids were estimated using the Ninhydrin method as described by Moore and Stein (1954). The activity of protease was estimated using the method of Ninhydrin as described by Davis and Smith (1954). The activities of alanine and aspartate amino transferases were estimated using the method of Reitman and Frankel (1957). Glutamate dehydrogenase (GDH) activity was estimated using the method of Lee and Lardy (1965). The level of ammonia was estimated by using the method of Bergmeyer (1965). The histological sections of tissues were taken by adopting the procedure as described by Humason (1972). The t-test was adopted to evaluate significance at 5% level.

RESULTS AND DISCUSSION
The survival ability of an animal to stress majorly depends on its protein synthetic potentials. Adrenal hormone insufficiency also significantly influences on the protein metabolism (Casadevall et al., 1999) leading to profound changes in structural organization and functional ability of different reproductive tissues of adrenalectomized rats. The maintenance of
proteins in highly organized state requires an active and continuous supply of energy. If this is impaired the organ structures breakdown and the proteins get partially denatured. The data on the total protein content presented in Table 1 and 2 revealed a significant decrease (P<0.05) of it in penis of male and vagina of female ADX rats at day 15 and day 30 of experimentation compared with the respective male and female SO rats. It indicates the breakdown of proteins under ADX stress. The breakdown could be due to the results of domination of proteolysis over synthesis under enhanced proteolytic activity and/ or decreased levels of gonadotropins and loss of weight of reproductive organs (Venkata Reddy et al., 2006). Narasimha Varma et al. (2007) reported a decrease in the level of total proteins in the testis of male rat under different stress conditions. The adrenalectomy also exerted stress on animals, and induced alterations in protein turnover leading to the depletion of total proteins in the reproductive tissues of ADX rats. Increase in protease activity and amino acid levels also indicate the turn over of proteins for metabolic reorganization. The amino acids released might to incorporate into TCA cycle for energy releasing purposes, as evident from the increase in AAT and AlAT activities to combat the energy crisis during ADX stress (Almon and Dubois, 1985). The intensity of the proteolytic activity was greater at day 30 than at day 15 in both the sex groups of ADX rats.

It could be due to more decrease in the levels of adrenal hormones in circulation. Higher magnitude of proteolysis in the vagina of female ADX rats than in males could result in greater weight of loss in them on adrenalectomy (Venkata Reddy et al., 2006). Probably cortisol hormone may greatly decrease in the circulation of female ADX rats than in male ADX rats. Increase in the activities of AAT, AIAT and GDH suggests active trans-deamination for the incorporation of ketoacids into the TCA cycle to favour gluconeogenesis for energy production. So, these enzymes function as a strategic link between carbohydrate and protein catabolisms and the changes in them can be considered as sensitive indicators of stress (Bag et al., 1999). In the present study slight elevation of ammonia observed in the penis of male and vagina of female ADX rats could be due to increased GDH activity and failure of liver function. It is known that profuse ammonia production also takes place through the operation of purine nucleotide cycle. The over all results suggest a severe proteolytic activity in the penis of males and vagina of female rats, more in females than in males on adrenalectomy and the severity increases with the duration of operation. Corresponding to the changes in protein levels, some degenerative changes in the histology are observed in the penis of male and vagina of female ADX rats relative to
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The differences between SO and ADX at both day 15 and 30 are statistically significant (P < 0.05). *denotes not significant with SO (P>0.05)

Table 2 : The levels of total proteins (TP), free amino acids(FAA), ammonia and the activities of protease, alanine amino transferase(AIAT), aspartate amino transferase(AAT) and glutamate dehydrogenase(GDH) in the vagina of SO and ADX female rats at day 15 and day 30 of experimentation. Each value is a mean of six individuals. The per cent decrease or increase over to SO is given in parenthesis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SO</th>
<th>ADX (day 15)</th>
<th>ADX (day 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (mg/g wet wt)</td>
<td>102.24±10.24</td>
<td>88.04±8.804</td>
<td>76.02±7.602</td>
</tr>
<tr>
<td>% Change</td>
<td>(-13.88)</td>
<td>(-25.64)</td>
<td></td>
</tr>
<tr>
<td>FAA (µM/g wet wt)</td>
<td>2.714±0.189</td>
<td>3.142±0.124</td>
<td>3.426±0.239</td>
</tr>
<tr>
<td>% Change</td>
<td>(+15.77)</td>
<td>(+26.23)</td>
<td></td>
</tr>
<tr>
<td>Protease (µM amino acid nitrogen/g wet wt)</td>
<td>0.989±0.037</td>
<td>1.098±0.043</td>
<td>1.253±0.050</td>
</tr>
<tr>
<td>% Change</td>
<td>(+11.02)</td>
<td>(+26.69)</td>
<td></td>
</tr>
<tr>
<td>Ammonia (µM/g wet wt)</td>
<td>1.118±0.044</td>
<td>1.190±0.047</td>
<td>1.350±0.054</td>
</tr>
<tr>
<td>% Change</td>
<td>(+6.44)</td>
<td>(+20.75)</td>
<td></td>
</tr>
<tr>
<td>AIAT (µM pyruvate/mg protein/h)</td>
<td>2.049±0.080</td>
<td>2.216±0.088</td>
<td>2.456±0.098</td>
</tr>
<tr>
<td>% Change</td>
<td>(+8.15)</td>
<td>(+19.86)</td>
<td></td>
</tr>
<tr>
<td>AAT (µM oxalo acetate/mg protein/h)</td>
<td>2.220±0.088</td>
<td>2.456±0.090</td>
<td>2.694±0.107</td>
</tr>
<tr>
<td>% Change</td>
<td>(+10.63)</td>
<td>(+21.35)</td>
<td></td>
</tr>
<tr>
<td>GDH (µM formozan/mg protein/h)</td>
<td>0.087±0.003</td>
<td>0.095±0.004</td>
<td>0.105±0.004</td>
</tr>
<tr>
<td>%</td>
<td>(+9.195)</td>
<td>(+20.689)</td>
<td></td>
</tr>
</tbody>
</table>

The differences between SO and ADX at both days 15 and 30 are statistically significant (P < 0.05).

controls (Fig. 1a-c and Fig. 2a-c). They provide support to the changes observed in protein levels. A mild damage is seen in areolar connective tissue and elastic fibres of penis of
rats at days 15 (Fig.-1b), on day 30, the three erectile tissues of penis overlapped with irregular arrangement and congestion of areolar tissue (Fig.-1c). The tissue cells appeared pyknotic and haemorrhagic spots are seen at few regions. These degenerative changes can be correlated to the decreased availability of glucocorticoids and testosterone in ADX rats (Nair et al., 1995). On day 15, the vagina of ADX rat showed less number of leucocytes and more space between fibres of fibrosa (Fig.-2b). At day 30 of ADX the vagina exhibited the presence of still less number of leucocytes in mucous, while the elastic fibres of musculares mostly disappeared and the vaginal gap was also greatly reduced. These changes less cornification reactions due to decreased level of estrogen in the circulation as reported by Butcher et al. (1974) that the sex steroids especially influence in the cyclical changes in various stages of estrous cycle lower level of them results in less cornification reactions. On the whole, the adrenalectomy causes an irrecoverable damage to the reproductive organs of male and female rats. The structural disorganization is considerably worse in female ADX rats than the males especially at day 30. So, decrease in protein levels and the corresponding histopathological changes in the rats on adrenalectomy are dependent on the sex of the animal and duration of the removal.

**Fig. 2:** (a) Transverse section of vagina of female sham operated (SO) rats (b) & (c) : Transverse section of vagina of female adrenalectomized rats (ADX) at day 15 and at day 30, respectively

**Fig. 1:** (a) Transverse section of penis of male sham operated (SO) rats (b) & (c) : Transverse section of penis of male adrenalectomized rats (ADX) at day 15 and at day 30, respectively

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