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A study on the protein metabolism and histology of the liver of male and female albino rates (*Rattus norvegicusalbinus*) on adrenalectomy

M. VENKATA REDDY[1] AND K. RADHAKRISHNAIAH[2]
[1]Department of Zoology,Yogi Vemana University, KADAPA (A.P) INDIA
[2]Department of Zoology, Sri Krishnadevaraya University, ANATAPUR (A.P) INDIA

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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(AlAT),aspartate amino transferase (AAT) and glutamate dehydrogenase(GDH) were observed in the liver of male and female rats on adrenalectomy (ADX) at day 15 and day 30 compared with sham operated (SO) rats. These results indicated significant proteolysis and active trans- deamination in the hepatic tissue of rats on adrenalectomy. Increase in protease activity and amino acid levels could be due to the turnover of proteins for metabolic reorganization. The magnitude of those changes were significantly more in the liver of female ADX rats than in the liver of males. It revealed more susceptibility of females to adrenalectomy than the males. Longer the duration of adrenalectomy more is 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 liver of male and female ADX rats at day 15 day 30 compared to normals. A mild degree of destruction of hepatocytes with darkened nuclei and widening of sinusoidal spaces in few regions were observed at day 15 and severe cytoplasmic and nuclear damage at day 30 in the liver of ADX males suggest that adrenalectomy leads to decreased protein synthesis. The female ADX rats showed widening of sinusoidal spaces in few regions at day 15 and on day 30 the nuclei of hepatocytes shrunken, while karyolysis is also observed in few nuclei. These changes support that female rats proved to more vulnerability on ADX stress than the male ADX rats. In conclusion the observations, indicated proteolysis and active trans-deamination to favour to gluconeogenesis for energy producction in the hapatic tissues of rats on adrenalectomy. It could lead to structural disruption and decreased metabolic ability that appeared more in females than in males on ADX, and in both the sexes it increased with the duration of adrenalectomy.

Key words : Albino rats, Adrenalectomy, Liver, Proteins, Histology

INTRODUCTION

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. 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 including enzymes (Assem and Hunke, 1983).

Removal of endocrine 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. Bilaterial removal of adrenal gland thus leads 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).

The liver is one of the most metabolically active tissues. It has important functions in the metabolism of all the three principle dietary constituents like proteins, carbohydrates and lipids. Increase in protein synthesis in the liver is an important action of the adrenal steroids on increased metabolic availability of aminoacids (Guyton and Hall, 2000). However, there are no reports available on the effect of adrenal dysfunction on the proteins and histology of the hepatic organ of rats. Hence, it is of interest in the present study to trace out the effect of adrenalectomy on protein levels and histology of liver of male and female albino rats.

MATERIALS AND METHODS

Healthy Wistar strain male and female albino rats (*Rattus norvegicus albinus*) of the age of 120 days and body weight 220±10g have been selected for present study. The selection of albino rats is based on their ability of survival, more withstanding capacity in a fairly wide range of stress conditions and easy maintenance and handling.

The stock of the litters was obtained from Indian Institute of Sciences, Bangalore. The rat colony was maintained in laboratory at 28±2ºC with 12 h light and 12 h of darkness. Rats were fed on standard rat diet obtained from Hindustan Lever Ltd., Bangalore, and water was supplied *ad libitum.*

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 liver of male and female rats were isolated for the estimation of the following parameters of protein metabolism. Histological sections in liver of male and female both ADX and SO rats were also taken.

The level of total protiens were estimated by Lowry *et al.* (1951). Free aminoacids were estimated by using the Ninhydrin method as described by Moore and Stein (1954). The activity of protease was estimated using the method 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

Proteins, the basic units of life, usually account for 68-85% of the dry matter of any growing animal (Joseph *et al.*, 1992). The survival ability of an animal to stress majorly depends on its protein synthetic potentials. Adrenal hormone insufficiency also significantly influences on the protein meabolism (Casadevall *et al.*, 1999) leading to profound changes in strucrural organization and functional ability of hepatic tissue of adrenalectomized rats. The maintenance of proteins in highly organized state requires an active and continous supply of energy. If this is impaired the organ structure breaksdown 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 liver of male and 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 synsthesis under enhanced proteolytic activity and/or decreased levels of adrenal hormones and loss of weight of hepatic organs (Venkata Reddy *et al.*, 2008). Bishayi and Ghosh (2003) stated that decreased total serum protein levels in circulation and also decreased levels of total protein content in the liver of ADX rats could be due to the variations in glucocorticoid levels. Below to physiological levels glucocorticoids exert profound effects on the synthesis of protein and enhance their break down (Quan and Walser, 1991). Decreased level of total protein content in the liver also reported in rat exposed to different stress condition (Bhandarkar and Khan, 2003). The adrenalectomy also exerted stress on animals and induced alterations in protein turnover leading to the depletion of total proteins in the liver of ADX rats. Increase in protease activity and amino acid levels also indicates the turn over of proteins for metabolic reorganization. The amino acids released might to incorporate into TCA cycle for energy releasing purposes, as evidenced by the increase 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 indicates that the survival ability of rats on adrenalectomy is less on prolonged period of time as noticed by high break down of tissue structure and more accumulation of free aminoacids in it. Moreover higher magnitude of proteolysis in the liver of female ADX rats than in males indicate that females are more prone to stress there by appears severe proteolysis in liver. Also the females being metabolically less active than in males, any metabolic disorders influence more in liver, which in turn might have caused more breakdown of the structural and soluble

Table 1 : The levels of total proteins (TP), free amino acids(FAA), ammonia and the activities of protease, alanine amino transferase(AlAT), aspartate amino transferase(AAT) and glutamate dehydrogenase(GDH) in the liver of SO and ADX male rats at day 15 and day 30 of experimentation

±: Standard deviation			P : Level of significance
Parameters	SO	ADX (days 15)	ADX (days 30)
TP (mg/g wet wt)	115.205±10.368	95.090±8.558	79.311±7.931
% Change		(-17.460)	(-31.156)
FAA (µM /g wet wt)	13.856±0.969	17.118±1.198	18.216±1.275
% Change		(+23.542)	(+31.466)
Protease (µM amino acid nitrogen/g wet wt)	1.526±0.061	1.834±0.073	2.113±0.084
% Change		(+20.183)	(+38.466)
Ammonia (µM /g wet wt)	6.317±0.252	7.046±0.281	8.114±0.324
% Change		(+11.540)	(+28.508)
AIAT (µM pyruvate/mg protein/h)	5.415±0.216	6.221±0.248	6.813±0.272
% Change		(+14.884)	(+25.817)
AAT (µM oxalo acetate/mg protein/h)	8.818±0.352	10.146±0.405	12.216±0.488
% Change		(+15.060)	(+38.534)
GDH (µM formozan/mg protein/h)	0.376±0.015	0.423±0.016	0.483±0.019
%		(+12.50)	(+28.457)

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

Table 2 : The levels of total proteins (TP), free amino acids(FAA), ammonia and the activities of protease, alanine amino transferase(AlAT), aspartate amino transferase(AAT) and glutamate dehydrogenase(GDH) in the liver of SO and ADX female rats at days 15 and days 30 of experimentation

±: Standard Deviation			P: Level of significance
Parameters	SO	ADX (days 15)	ADX (days 30)
TP (mg/g wet wt)	119.587±11.958	84.401±8.440	70.236±7.023
% Change		(-29.422)	(-41.267)
FAA (µM /g wet wt)	10.059±0.704	14.357±1.004	16.047±1.123
% Change		(+42.727)	(+59.528)
Protease (µM amino acid nitrogen/g wet wt)	1.782±0.071	2.334±0.093	2.694±0.107
% Change		(+30.976)	(+51.178)
Ammonia (µM /g wet wt)	6.231±0.249	7.728±0.309	9.038±0.361
% Change		(+24.025)	(+45.048)
AIAT (µM pyruvate/mg protein/h)	5.721±0.271	7.371±0.294	7.962±0.318
% Change		(+28.841)	(+39.171)
AAT (µM oxalo acetate/mg protein/h)	9.826±0.392	12.948±0.517	14.918±0.596
% Change		(+31.772)	(+51.821)
GDH (µM formozan/mg protein/h)	0.368±0.014	0.473±0.019	0.520±0.021
%		(+28.532)	(+41.304)

The differences between SO and ADX at both day 15 and 30 are statistically significant ($P < 0.05$).
Each value is a mean of six individuals. The per cent decrease or increase over to SO is given in parenthesis

proteins of it.

Increase in the activities of AAT, AlAT 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 elevation of ammonia observed in the liver of male and female ADX rats could be due to increased GDH activity and failure of liver function. It is known the profuse ammomia production also takes place through the operation of purine nucleotide cycle. The over all results suggest a severe proteolytic activity in the liver

of males and 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 were observed in the liver of male and female ADX rats relative to controls (Fig. 1a-c and Fig. 2a-c). They provide support to the changes observed in protein levels. A mild degree of destruction of hepatocytes with darkened nuclei and widening of sinusoidal spaces in few regions were observed at day 15 in the liver of ADX male rat (Fig.-1b) and severe cytoplasmic and nuclear damage at day 30

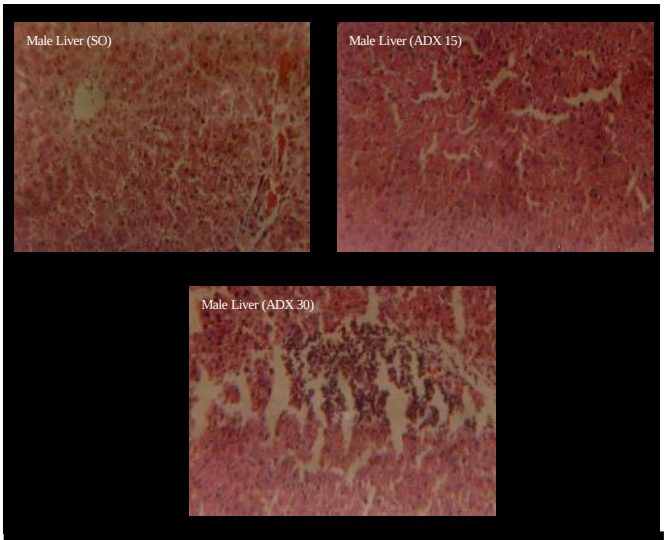

Fig. :1 (a): Transverse section of liver of male sham operated (SO) rats
(b) & (c): Transverse section of liver of male adrenalectomized rats (ADX) at day 15 and at day 30, respectively

(Fig. 1c) suggest that adrenalectomy leads to decreased protein synthesis. On day 15, the female ADX rats liver showed widening of sinusoidal spaces in few regions while the central vein was filled with debris (Fig. 2b). But greater degeneration was observed at day 30 where in the nuclei of heoatocytes shrunken, some of them exhibited more vacuolization (Fig.-1c). Udita Gubrelay *et al.* (2004) reported similar changes in hepatic tissues of male and female rats under different stress conditions. Female rats proved to more vulnerability on ADX stress than the male ADX rats. In conclusion the observations, indicated proteolysis and active trans-deamination to favour to gluconeogenesis for energy production in the hapatic tissues of rats on adrenalectomy. It could lead to structural disruption and decreased metabolic ability. The whole, the adrenalectomy causes an irrecoverable damage to the hepatic organ of male and female rats. The structural

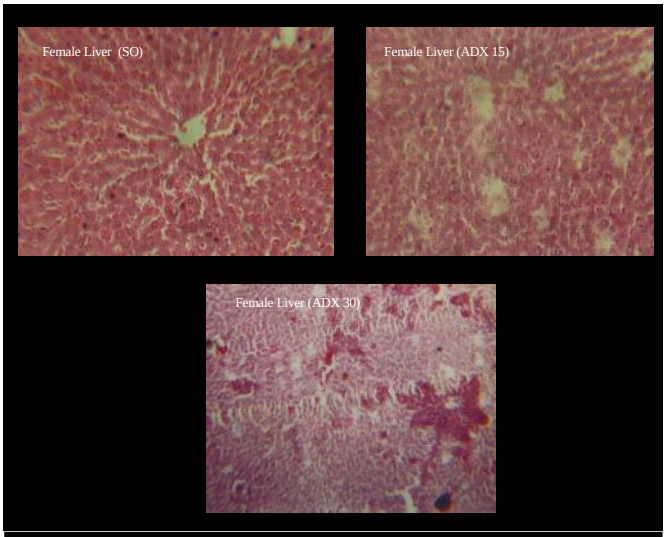

Fig.-2 (a): Transverse section of liver of female sham operated (SO) rats
(b) & (c): Transverse section of liver of female adrenalectomized rats (ADX) at day 15 and at day 30, respectively.

disorganization is considerably worse in female ADX rats than the males especially at day 30. So, the decrease in protein levels and corresponding histopathological changes in the rats on adrenalectomy are dependent on the sex of the animal and duration of the removal.

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