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Gender dependent effect of adrenaectomy on the energetics in the epididymis of male and uterus of female albino rats (*Rattus norvegicus albinus*)

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A decrease in the levels of glucose, glycogen and the activity of succinate dehydrogenase (SDH) along with an increase in the levels of lactate, pyruvate and the activity of lactate dehydrogenase (LDH) was observed in the epididymis of male and uterus of female rats on adrenalectomy (ADX) when compared with sham operated (SO) rats at days 15 and days 30 of experimentation. These results indicated suppression of oxidative metabolism and elevation of anaerobic glycolysis in the reproductive tissues of the male and female rats on adrenalectomy leading to the decrease in their energy levels. The magnitude of decrease, however, was significantly more in the uterus of female ADX rats than in epididymis of male ADX rats. And in both the animal groups the degree of decrease in the level of energetics was more at days 30 than at days 15. The over all results suggest that the impact of adrenalectomy on energetics was more in females than in males and it increases with the duration of experimentation.

Key words : Albino rats, Adrenalectomy, Epididymis, Uterus, Energetics.

INTRODUCTION

The classic endocrine glands are the pituitary, adrenals, thyroid, parathyroids, pancreatic islets, gonads and placenta. Adrenal gonadal interaction appears to depend upon overlapping function of the steroid hormones, relationship between reproductive function, stress and a variety of other mechanisms (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. Carbohydrates one of the major sources of immediate energy is essential for the reproductive activities (Gillian and Bell, 1982). The involvement of carbohydrate metabolism in the supply of energy to the reproductive process depends on the level of gonadotrophins and gonadal hormones (Venkata Reddy *et al.*, 2007). The existing literature indicates that ADX have influence on the hormonal integration of the body involving changes in the blood constituents and also tissues constituents. The changes witnessed in the hormonal and biochemical profiles of the serum during adrenalectomy might exhibit changes in the reproductive system and reproductive performance of animals.

Epididymis is an important organ in the male reproductive system. It provides conducive microenvironment by rapidly eliminating the harmful metabolic byproducts and free radicals (Hinton *et al.*, 1996). Epididymal spermatozoa are extremely vulnerable to oxidative stress. To overcome this problem epididymis has a rich source of an antioxidant enzyme that scavanges any excess reactive oxygen metabolite released by spermatozoa during epididymal transit (Dacheux *et al.*, 2003). Principal cells have been shown to be actively involved in the physiological functions of the epididymis involving in endocytosis, secretion and degenerative changes in epididymis of ADX rats (Nair *et al.*, 2002).

The uterus is an estrogen dependent organ and its structure and function dramatically changes with estrogen (Hadley, 2000). Uterus plays an important role in regulation of ovarian function and maintenance of normal reproductive cyclicity. Uterine weight decreased in ADX rats (Venkata Reddy *et al.*, 2007). But, the effect of adrenalectomy on the energetics in the uterus and epididymis are not well reported. Hence, the present study is taken up to understand the impact of adrenalectomy on energetics in different sex of rats at different experimental days on those tissues.

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±2°C 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 *adlibitum*.

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 Stith *et al.* (1989) and these two group of animals were considered as experimentals; one was maintained for 15 days and the other 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). After the stipulated period the epididymis in males and uterus in females were isolated for the estimation of the following parameters of carbohydrate metabolism in both ADX and SO rats.

The level of glucose was estimated by colorimetric method as described by Nelson and Somogyi (1952). Glycogen was estimated by using the anthrone reagent method as described by Caroll *et al.* (1956). The level of pyruvate was estimated using the method of Friedman and Hangen (1942). The level of lactate was estimated using the method of Huckabee (1961). Succinate dehydrogenase (SDH) activity was estimated using the colorimetric method described by Nachlas *et al.* (l960). Lactate dehydrogenase (LDH) activity was estimated by using the method of Srikantan and Krishnamoorthi (1955). The t-test was adopted to evaluate significance at 5% level.

RESULTS AND DISCUSSION

From the data presented in Table l and 2, it is seen that relative to sham operated the levels of glucose and glycogen decreased with the increase in the levels of pyruvate, lactate and the activities of LDH, in the organs of epididymis of male and uterus of female ADX rats at day 15 and day 30 of experimentation. It suggests the degradation of glycogen reserves in response to adrenal hormone insufficiency. The depletion of glycogen levels in reproductive tissues might be due to stepped up glycogen breakdown through glycogenolysis or glycolysis and/or decreased glycogenesis. It can be attributed that the decreased glycogenesis might be responsible for the reduced glycogen content on adrenalectomy. The decrease in glucose content in male epididymis and uterus of female ADX rats than in SO rats also envisages decreased level of cortisol in circulation and enhanced utilization of it to meet the energy demands of tissues. In consonance to the present study ADX rats showed hypoglycemia and increased uptake of glucose (Bady *et al.*, 2002). In between the two sex groups, the level of glucose and glycogen decrease was more in the uterus of female ADX rats than the epididymis of male ADX rats at 15 and 30 days of experimentation. Blair *et al.* (1996) reported the brown adipose tissue was formed in female ADX rats due to increased lipid synthesis during adrenal hormone insufficiency. Consequently less is the synthesis of glycogen and more glucose utilization in females than the males.

The increase in the levels of pyruvate and lactate in reproductive tissues of male and female ADX rats with the duration indicates the existence of anaerobiosis or hypoxia in them. The effect of adrenalectomy on testicular LDH activity studied by Valivullah *et at.* (1983) reported a general increase of it leading to the formation of lactic acid from pyruvate, which also supports for the elevation of lactic acid in epididymis of male and uterus of female rats. Another reason for the elevated lactic acid might be the possible influx of it from the blood into the reproductive tissues due to the impaired function of plasma membrane. Anitha and Indira (2006) reported increased LDH activity in epididymis under various stress conditions in albino rats. The adrenalectomy also exerts stress condition in animals which in turn might have been responsible for the elevation of LDH activity in the epididymis and uterus on ADX rats. Epididymis and uterus being the reproductive tissues in male and female rats, respectively, the elevation in pyruvic acid may not only be due to the breakdown of glucose but also from other biochemical precursors such as glycerol, which mobilizes towards glycolysis under various stress conditions (Savina and Wojtczak, 1977).

The decreased level of SDH activity in the epididymis of male and uterus of female ADX rats indicates decreased gonadal hormones in circulation, because this enzyme activity is gonadal hormone dependent (Connell, 1972). Venkata Reddy *et al.* (2007) revealed that the enzymes concerned with glucose metabolism are altered by adrenalectomy and proved that they are under the

Table 1 : The levels of tissue glucose, glycogen, pyruvate and lactate and the activities of lactate dehydrogense (LDH), succinate dehydrogenase (SDH) in the epididymis of SO and ADX male rats at day 15 and day 30 of experimentation

±Standard deviation — P: Level of significance

Parameters	SO	ADX (day 15)	ADX (day 30)
Glucose (mg/g wet wt)	1.654±0.066	1.456±0.058	1.285±0.051
% Change		(-11.970)	(-22.309)
Glycogen (mg/g wet wt)	2.896±0.112	2:51 0±0.l 00	2.283±0.091
% Change		(-13.328)	(-21.167)
Pyruvate (mg/g wet wt)	2.69l±0.l07	3.116±0.124	3.395±0.135
% Change		(+15.793)	(+26.161)
Lactate (mg/g wet wt)	2.20l±0.088	2.434±0.097	2. 706±0.l 08
% Change		(+10.586)	(+22.944)
LDH (μM formazan/mg protein/h)	1.008±0.040	1.116±0. 044	1.224±0.048
% Change		(+10.714)	(+21.428)
SDH (μM formazan/mg protein/h)	0.492±0.0 19	0.430±0.017	0.374±0.014
% Change		(-12.601)	(-23.983)

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 over to SO is given in parenthesis

Table 2 : The levels of tissue glucose, glycogen, pyruvate and lactate and the activities of lactate dehydrogense (LDH), succinate dehydrogenase (SDH) in the uterus of SO and ADX female rats at day 15 and day 30 of experimentation

±Standard deviation — P: Level of significance

Parameters	SO	ADX (day 15)	ADX (day 30)
Glucose (mg/g wet wt)	1.928±1:0.077	1.440±0.057	1.202±0.048
% Change		(-25.311)	(-37.655)
Glycogen (mg/g wet wt)	1.832±0.073	320±0.052	1.180±0.051
% Change		(-27.947)	(-35.589)
Pyruvate (mg/g wet wt)	2.499±0.099	3.254±0.130	3.568±0.142
% Change		(+30.212)	(+42.777)
Lactate (mg/g wet wt)	2.024±0.080	2.505±0.100	2.798±0.111
% Change		(+23.764)	(+38.241)
LDH (μM formazan/mg protein/h)	0.946±0.037	1.156±0.046	1.253±0.050
% Change		(+22.198)	(+32.452)
SDH (μM formazan/mg protein/h)	0.396±0.015	0.303±0.012	0.258±0.010
% Change		(-23.484)	(-34.848)

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 over to SO is given in parenthesis.

control of adrenal hormones. This is correlated with excessive oxidation of glucose and decreased gluconeogenesis from the body (Narasimha Varma *et al.*, 2007). During physiological stress condition diversion of phosphoenol pyruvate leads to increased formation of fumarate resulting in the inhibition of SDH (Chinoy *et al.*, 1996). However, significantly greater inhibition of SDH activity in female ADX rats at both the days *i.e.* 15 and 30 (day 15 < day 30) when compared with male ADX rats at said experimental days indicate female ADX rats are more sensitive to ADX stress than the males. It could be due to more suppression of oxidative metabolism and more accumulation of lactic acid was formed in female ADX rats over to male ADX rats. Female rats are more vulnerable than the males under ADX stress. This is reason for the female rats are more sensitive to adrenalectomy than the males (Venkata Reddy *et al.*, 2008). Thus the energetic efficiency on adrenalectomy is sex and time dependent.

On the whole, the impact of adrenalectomy increased with the duration as indicated by the progressive prevalence of anaerobic glycolysis and suppression of oxidative metabolism in the epididymis of male and uterus of female ADX rats from day 15 to

day 30. Hence, the effect of adrenalectomy on energetics is not only dependent on the sex of rats but also on duration of stress.

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