Journal of Applied and Natural Science 1(1): 62-65 (2009)

JANS

Alterations in carbohydrate metabolism under cryptorchid condition in albino rats

K. Narasimha Varma, E. Madhuri, S. Sadak Basha, YR. Radha Madhavi, M Guru Sekhar and M. Bhaskar*

Department of Zoology, Sri Venkateswara University, Tirupati-517502 (A.P.), INDIA
*Corresponding author. E-mail: matchabhaskar@yahoo.com

Abstract : Bilateral cryptorchidism was induced surgically in adult wistar strain albino rats and the carbohydrate metabolic pathway has been studied in testis, and sex accessory organs of both control and cryptorchid animals by estimating the marker enzymes and the substrates of the metabolism. In cryptorchid animal tissues, accumulation of lactic acid and glycogen was observed with inhibited phosphorylase activity in comparison to the controls. The reproductive tissues like testis, epididymis, prostate gland and seminal vesicles had shown remarkable elevation in the glycogen content, which can be attributed to decreased phosphorylase activity. In view of androgen dependent nature of phosphorylase its inhibition can be correlated to decreased testosterone circulation in the body. Consequently the free glucose content of the tissues was markedly decreased suggesting a decrease in the mobilization of the carbohydrates into energy metabolism. All the reproductive tissues had shown significant accumulation of lactic acid with inhibited oxidative enzyme activities. Thus the reproductive tissue oxidative metabolism had been suppressed during cryptorchidism leading to a shift towards glycolysis and creating a situation of functional suppression.

Keywords: Cryptorchidism, Carbohydrates, Reproduction, Enzyme, Rat testis

INTRODUCTION

Cryptorchidism represents one of the most common disorders in man. Cryptorchdism and elevation of testicular temperature disturb the biochemical activities (Main and Waite, 1977). Changes in the hormonal profiles and altered cellular functions in the atrophied tubules might reflect on the metabolic activities of damaged testis (Hejmej and Biliñska, 2008). The altered metabolism, as a result of increased temperature might be due to impairment of spermatogenesis and it was also suggested that the spermatogenic arrest was because of reduced levels of substrate availability to the tissue (Ewing and Van Demark, 1963; Hilczer *et al.*, 2008). In rat testis, glucose is an essential substrate for the maintenance of tissue integrity (Mancine *et al.*, 1960), and ATP production (Means and Hall, 1968). The cytochemical behavior of the cryptorchid testis may reflect functional disturbances in the carbohydrate metabolism (Seilicorich and Lloret, 1973; Hilczer *et al*, 2008). Changes in glucose metabolism of rat testis after translocation to the body cavity have been followed for up to 44 days (Free *et al.*, 1969). The fall in testes weight was accompanied by a parallel fall in glucose-^{14}C oxidation to $^{14}CO_2$ from glucose-1-^{14}C and indicated a concomitant change in pentose cycle activity. All these changes appear to be associated with loss of germinal cells and emergence of a pattern of metabolism reflecting that of surviving cells i.e. interstitial and sertoli cells.

Farooqui *et al.* (1997) suggested that the degenerative changes in abdominal testis may be associated with decreased glut-3-mediated glucose transport in semniferous tubules and spermatogonia. Though considerable work has been carried out on testis under cryptorchidism, very scanty information is available on sex accessory organs. Hence, the present study has been undertaken to understand the possible effect of cryptorchidism on the glycolysis of reproductive tissues viz testis, epididymis, prostrate gland and seminal vesicles.

MATERIALS AND METHODS

Adult male wistar strain albino rats weighing about 125 ± 5g and age 100 ± 5 days were selected for experimentation and they were divided into 2 batches. Each batch consisted of 8 rats. The first batch was treated as control and the second as experimental. The control rats were anaesthetized with anesthetic ether, perineal area shaved, swabbed with 95% ethanol. One cm long bilateral incision was made at the level of the inguinal canals, sutured and treated as sham operated. The second batch rats were

anaesthetized with anaesthetic ether and in each case one cm long bilateral incision was made at the level of inguinal canals and testes were carefully translocated into abdominal cavity with blunt forcep. Care was taken to avoid damage to the testicular artery. Both the inguinal canals were sutured with silk thread to prevent the descent of the testis from abdominal cavity (Ewing and Schanbucher, 1970). The experimental and sham operated rats were maintained at laboratory conditions (26 ± 2°C and 12 hrs of light and 12 hrs of darkness), fed on standard rat diet (Hindustan Lever Ltd., Mumbai) and water was supplied ad libitum for a period of 60 days. On 61st day, the animals were weighed and sacrificed by cervical dislocation ;and the testis and sex accessory organs like epididymis, ventral prostate and seminal vesicles were isolated carefully, weighed, chilled rapidly in ice box and utilized for biochemical analysis.

Glucose (Mendel *et al.,* 1954), glycogen (Kemp and Van Heijningen, 1954), lactic acid (Barker and Summerson, 1941, as modified by Huchabee, 1961), pyruvic acid (Friedman and Hangen, 1942), aldolase activity (Bruns and Bergmeyer, 1965), phosphorylase activity (Cori *et al.,* 1955) were estimated in the testis and sex accessory organs.

RESULTS

Testis and all the sex accessory organs viz epididymis, prostate gland and Seminal vesicles had shown significant accumulation of glycogen and lactic acid under cryptorchid condition over the control. Free glucose content of the testis and all sex accessory organs were highly depleted from the control values. The activity levels of phosphorylase 'a' were suppressed and phosphorylase 'b' were elevated in cryptorchid condition over the control in all the reproductive tissues studied. The aldolase activity levels were suppressed in all the reproductive tissues of cryptorchid animals than control. The pyruvic acid levels were depleted in all the reproductive tissues of cryptorchid condition over control.

DISCUSSION

The carbohydrate metabolism leading to glycolysis was studied in all the diabetic male reproductive tissues of the rat under cryptorchid condition. Interestingly, all the reproductive tissues had significant accumulation of lactic acid, suggesting impairment in the glycolytic phase of the carbohydrate metabolism in the reproductive tissues of the cryptorchid animal. This type of pattern where the tissues have switched over to anaerobic metabolism might be responsible for over all accumulation of glycogen in all the tissues.

The testis showed significant accumulation of glycogen which reveals either increased glycogenesis or decreased glycogenolysis and/or glycolysis of the tissue. Phosphorylase activity, which is a marker for glycogenolysis showed significant suppression in the cryptorchid testis, suggesting that the abdominal position of the testis was inducing inhibition on the glycogenolysis. Thus accumulation of glycogen in the tissue seems to be due to decreased glycogenolysis. Since, phosphorylase activity has been androgen dependent (Chinoy and Sheth, 1977), such a suppression in the enzyme activity can be related to decreased testosterone levels in circulation in the body (Boujard *et al.,* 1995). However the free glucose content of the testis was decreased conspicuously. Which can be related to decreased mobilization from glycogen (Wu and Murono, 1996). Phosphorylase activity has been shown to be inhibited by lactic acid which exerts feed back inhibition on the enzyme. Thus accumulation of lactic acid in the tissue seems to be another reason for inhibited phosphorylase activity of the cryptorchid testis, because lactic acid is an allosteric inhibitor of phosphorylase activity. Consequently, the tissue had showed depleted glucose content which will be vital for the testicular function, namely spermatogenesis and androgenesis (Mancine *et al.,* 1960). Thus testicular carbohydrate metabolism seems to be forming a limiting factor for its function under cryptorchid condition. Aldolase activity was inhibited with depleted level of pyruvic acid suggesting the possibility of decreased metabolic activities of the cryptorchid testis. Lactic acid was accumulated remarkably suggesting a metabolic shift in the testicular carbohydrate metabolism towards anaerobic phase, which will be deleterious to the functions the testis.. Thus, the testicular tissue under abdominal position had induced metabolic modulations which were non-congenial towards spermatogenesis as well as androgenesis (Hejmej and Biliñska, 2008). The sex accessory organs such as epididymis, prostate gland and seminal vesicles also exhibited the same pattern of carbohydrate metabolism as like as in the testis under cryptorchid condition.

Epididymis showed a conspicuous increase in the glycogen content with inhibited phosphorylase activity suggesting the acceleration of glycogenesis pathway in the tissue. The phosphorylase activity is oriented towards inactive condition owing to increase in the inactive phosphorylase activity. The free glucose content was depleted suggesting low level of energy substances like glucose in the tissue. Aldolase activity was inhibited with increased lactic acid content and depleted pyruvic acid content was observed in the cryptorchid epididymis. This type of inactive condition of the metabolism was witnessed in the epididymis under cryptorchid condition, which indicates the inefficient functional condition of epididymis. Since epididymis was involved in sperm maturation as well as storage (Conglio *et al.,* 1975; Delpio and Raisman, 1978), these two functions seem to be inhibited in the tissue, which can be attributed to the

Table 1. Effect of experimental bilateral cryptorchidism on glycogen, glucose lactic acid, pyruvic acid contents and activity levels of phosphorylase 'a' (active), phosphorylase 'b' (Inactive) and aldolase of Testis, Epididymis, Prostate gland and Seminal vesicles. (Each value represents the mean ± SD of six individual observations, + % increase, - % decrease respectively over sham operated control given in parenthesis. * level of significance (P< 0.001), C= Control, E= Experimental).

	Tissue							
	Testis		Epididymis		Prostate Gland		Seminal vesicles	
Parameter	C	E	C	E	C	E	C	E
Glycogen (mg/g fresh tissue)	1.86 ±0.04	3.56* ±0.09 (+91.39)	0.521* ±0.036	0.801* ±0.028 (+53.74)	3.45 ±1.20	5.16 ±0.87 (+49.56)	0.836 ±0.037	1.31 ±0.061 (+57.06)
Glucose (mg/g fresh tissue)	13.21 ±0.32	5.26* ±0.210 (-60.18)	3.17* ±0.0151	1.48* ±0.123 (-53.28)	6.91 ±1.34	3.80 ±0.92 (-44.96)	5.18 ±1.12	2.51 ±1.07 (-51.52)
Lactic acid (mg/g fresh tissue)	27.85 ±1.28	39.54* ±1.17 (+41.97)	16.44* ±0.59	21.13* ±0.74 (+28.53)	20.45 ±1.22	26.67 ±1.18 (+30.64)	21.92 ±0.981	30.24 ±1.02 (+37.95)
Pyruvic acid (µmoles/ g fresh tissue)	33.18 ±0.071	19.10* ±0.052 (-42.43)	28.42* ±1.19	22.46* ±1.26 (-20.97)	21.21 ±1.28	14.41 ±1.07 (-32.06)	28.13 ±1.17	19.11 ±1.28 (-32.06)
Phosphorylase "a" (µmoles of pi. formed/ mg protein/h)	0.432 ±0.03	0.177* ±0.02 (-59.03)	1.46* ±0.27	1.04* ±0.523 (-28.87)	1.28 ±0.051	0.530 ±0.047 (-58.60)	1.33 ±0.71	0.862 ±0.682 (-35.33)
Phosphorylase "b" (µmoles of pi. formed/ mg protein/h)	0.697 ±0.02	1.15* ±0.06 (+65.28)	0.238* ±0.071	0.357* ±0.084 (+50.00)	0.775 ±0.024	1.14 ±0.027 (+46.45)	0.925 ±0.043	1.18 ±0.039 (+27.90)
Aldolase (µmoles of FDP cleared/mg protein/h)	1.45 ±0.04	0.399* ±0.04 (-72.39)	1.32* ±0.072	0.531* ±0.064 (-59.80)	1.84 ±0.481	1.07 ±0.721 (-41.90)	1.34 ±0.071	0.535 ±0.079 (-59.92)

decreased circulation of testosterone in the body under abdominal condition of the testis (Wu and Murono, 1996). Both seminal vesicles and prostate gland were known to contribute seminal plasma under normal conditions of the reproduction. These glands have been known to produce fructose, sorbitol and free glucose into the seminal plasma for the maintenance of sperms. These glands had showed elevated glycogen content with accumulation of lactic acid and inhibited glycolytic pathway during cryptorchidism. Even then the sex accessory glands have been maintained under inactive state possibly because of less availability of testosterone under cryptorchidism. Such a situation can be comparable to the orchectomy where the male gonads were excised. Thus under cryptorchid conditiopn, the testis, though available in the body was unable to maintain active functional sex accessory system which probably owing to inhibited androgen synthesis.

REFERENCES

Barker, S.B. and Summerson, W.H. (1941). The colorimetric determination of lactic acid in biological material. *J.Bio.Chem.*138:535.

Boujard, N., Hocheru, M.T., De Reviers, P., Kamtchouing, C., Perream and Cerreran, S. (1995). Evolution of somatic and germ cell populations affer buslfan treatment in utero or neonatal cryptorchidism in the rat. *J. Andrologia*. 27 (4). 223-228.

Bruns, F.H. and Bergmeyer, H.U. (1965). Fructose1, 6-diphosphate aldolase, In: Methods of enzymatic analysis. H.U. Bergmeyer (ed), Academic press, New York, 724.

Chinoy, N.J. and Sheth, K.M. (1977). Effects of $CdCl_2$ on the secretory functions of sex accessory glands of rats. *Ind. J. Exp. Biol.*15:594-597.

Conglio, J.G., Grogan, W. M Jr. and Rahampy, R.K. (1975). Lipid and fatty acid composition of human testes removed at autopsy. *Bio. Reprod.*, 12: 255-259.

Cori, G.T., Illingworth, B.B., and Keller, P.G. (1955). Muscle phosphorylase In: Methods in enzymology. S.P. Colowick and N.O. Kaplan (eds), Academic press. New York.. Vol-I, 200.

Delpio, A.G. and Raisman, R. (1978). C-AMP in spermatozoa taken from different segments of the rat epididymis. *Experentia*, 34(5): 670-671.

Ewing, L.L. and Van Demark, N.L. (1963). Factors affecting testicular metabolism and function. II. Effect of temperature elevation *in vivo* on subsequent metabolism activity of rabbit testicular tissue *in vitro*. *J. Reprod. Fertil.*, 6: 9.

Ewing. L.l. and Schanbecher. L. M. (1970). Effects of

experimental cryptorchidism on the activity of selected enzymes in rat testis. *J of Endocrinology*. 87, 129.

Farooqui, S.M., Al-Bagdadi, F. O., Donnel, J.M. and Stout, R. (1997). Degenerative changes in spermatogonia are associated with loss of glucose transporter (Glut 3) in abdominal tetis of surgically induced unilateral cryptorchidism in rats. *Biochem. Biophys. Res. Commun.*, 18: 236 (2); 407-12.

Free, M.J., Massive, E.D. and Van Demark, N.L. (1969). Glucose metabolism by the cryptorchid rat testis. *J. Biol. Reprod.*, 1: 354.

Friedman, T.E. and Hangen, G.E. (1942). Pyruvic acid I. Collection of the blood for determination of lactic acid. *J. Biol. Chem.* 147: 67,

Hejmej, A. and Biliñska, B. (2008). The effects of cryptorchidism on the regulation of steroidogenesis and gap junctional communication in equine testes. *Endokrynol Pol*., 59(2):112-8.

Hilczer, M., Smyczyñska, J., Stawerska, R. and Lewiñski, A. (2008). Effects of one-year low-dose growth hormone (GH) therapy on body composition, lipid profile and carbohydrate metabolism in young adults with childhood-onset severe GH deficiency confirmed after completion of growth promotion. *Endokrynol Pol*., 59(4):292-300.

Huchabee (1961). *Hawk's physiological chemistry.* 14th Edition. Tata Mc Graw –Hill publishing Co. Ltd., New Delhi, pp. 55-56.

Kemp, A. and Van Heijningen, M.K. (1954). A colorimetric micro method for the determination of glycogen in tissue. *Bio. Chem. J*., 56: 646.

Main, S.J. and. Waite, G.M.H. (1977). The blood testis barrier and temperature damage to the testis of rat. *J. Reprod. Fertil.*,51: 439-450.

Mancine, R.E., Penhos, J.C., Izquierdo, I.A. and Heinrich, J.J. (1960). Effects of acute hypoglycemia on rat testis. *Proc. Soc. Exp. Biol. Med*.,104: 699.

Means, A.P. and Hall, P.F. (1968). Protein biosynthesis in the testis: II Role of adenosine triphosphate (ATP) in stimulation by glucose. *Endocrinology*, 82: 86.

Mendel, B., Kemp, A. and Myers, D.K. (1954). A colorimetric micro method for the determination of glucose. *Biochem. J*. 56: 639.

Seilicovich, A. and Lloret, A.P.(1973). Glycogen and related enzymes in normal and cryptorchid human testis: A cytochemical study. *J. Reprod. Fertil*., 33: 379.

Wang, Z.Q., Wetanabe, Y., Toki, A. and Itano, T. (2002). Altered distribution of sertoli cell vimentin and increased apoptosis in cryptorchid rats. *J. Pedietrosury*. 37 (4): 648-652.

Wu, N. and Murono, E.P. (1996). Temperature and germ cell regulation of leidig cell proliferation stimulated by sertolicell mitogenic factor, possible role in cryptorchidism. *J. Andrologia*, 28 (5): 247-257.