EFFECT OF BILATERAL VASECTOMY ON TESTICULAR METABOLISM IN ALBINO RATS

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(Received on August 8, 1982)

Summary : The adult Wistar strain albino rats were vasectomised by conventional method and maintained for six months. The vasectomized rat testis had elevated water content with depleted dry matter. Glycogen content was increased with indication of mobilization of hexoses into HMP pathway. The vasectomized rat testis showed preferential utilisation of triglycerides. In view of increased 3 β–HSD and 17 β–HSD activities, accelerated androgenesis was envisaged in vasectomized rat testis.

Key words : vasectomy testis leydig cells androgenesis glycogen phospholipids cholesterol

INTRODUCTION

Vasectomy has been employed as an important male contraceptive *device*. But a number of side effects have been reported both in man and animals (6, 23). There are several conflicting reports regarding the effect of vasectomy on the male reproductive system (16, 20, 23) while some investigators reported impaired androgenesis (20, 23), the others suggested improved androgenesis and leydig cell proliferation (24, 11, 20). Hence, an attempt has been made in the present study to evaluate the possible side effects of vasectomy on general metabolism of testis.

MATERIAL AND METHODS

Healthy Wistar strain adult albino rats of 145$\pm$2 days of age and weighing 250$\pm$5 *gms* were used for the present study. Rats were divided into two groups and one

group of rats were subjected to bilateral vasectomy by conventional method through abdominal approach as suggested by previous investigators (21) and the rats were maintained as per standard procedures. The other group of animals were sham operated and taken as controls. After six months of vasectomy both control and experimental animals were autopsized by cervical dislocation and the testes were immediately excised. The wet weight of tissue was determined and used for further analysis. The following estimations carried out using standard procedures. Tissue somatic indices, water content and dry matter were estimated gravimetrically. The glycogen (12), glucose (15), sorbitol dehydrogenase (18), glucose–6–phosphate dehydrogenase (2), total lipids (8), triglycerides, free fatty acids and cholesterol (19), glycerol (5), phospholipids (4), lipase activity (10), 3 β-Hydroxy Steroid Dehydrogenase (3 β-HSD) (3), and 17 β-Hydroxy Steroid Dehydrogenase (17 β→HSD) (3) were estimated.

RESULTS

TSI of the testis after vasectomy remained unaffected (Table I). While the water content was elevated, the dry matter was significantly decreased. The glycogen content was drastically elevated with decrease in free glucose level. The activity levels of sorbitol dehydrogenase and G–6PDH were considerably increased. The total lipid content was increased in vasectomized rat testis over the control level (Table II). The

TABLE I : The levels of tissue somatic indices, water content, dry matter, glycogen, glucose and activity levels of sorbitol dehydrogenase, and G-6-PDH in testis of normal and vasectomized rats.

S No.	*Component*	*TESTIS*		*Level of significance*
		Control	*Experimental*	
1.	Tissue somatic indices (TSI)	0.961±0.21	1.015±0.25	NS
2.	Water content (*mg/g* wet wt)	785.26±12.5	831.90±10.40	P<0.001
3.	Dry matter (*mg/g* dry wt)	214.74±26.46	168.1±18.3	P<0.01
4.	Glycogen (*mg/g* wet wt)	0.055±0.005	0.175±0.01	P<0.001
5.	Glucose (*mg/g* wet wt)	0.279±0.01	0.259±0.02	P<0.05
6.	Sorbitol dehydrogenase (*μmol* formazan formed/*mg* protein/h)	0.801±0.037	1.11±0.025	P<0.001
7.	G-6-PDH (*umol* formazan formed *mg* protein/h)	1.065±0.054	1.567±0.078	P<0.001

Note : Mean±S.D., values are mean of six observations.

TABLE II : The levels of total lipids, triglycerides, glycerol, free fatty acids, phospholipids, cholesterol and activity levels of 3 β–Hydroxy steroid dehydrogenase (3 β–HSD) and 17 β–Hydroxy steroid dehydrogenase (17 β–HSD) in testis of normal and vasectomized rats.

S. No.	*Component*	*TESTIS*		*Level of significance*
		Control	*Experimental*	
1.	Total lipids (*mg/g* wet wt)	81.32±2.84	89.79±2.61	P<0.001
2.	Triglycerides (*mg/g* wet wt)	18.14±1.88	11.37±2.14	P<0.001
3.	Lipid esterase (*μmoles* of PNPA Cleaved/*mg* protein/h)	0.432±0.041	0.613±0.052	P<0.001
4.	Glycerol (*mg/g* wet wt)	1.64±0.08	1.96±0.16	P<0.001
5.	Free fatty acids (*mg/g* wet wt)	29.12±3.31	20.18±2.98	P<0.001
6.	Phospholipids (*mg/g* wet wt)	27.46±4.63	36.13±4.37	P<0.02
7.	Cholesterol (*mg/g* wet wt)	3.602±0.14	8.32±1.31	P<0.001
8.	3 β-HSD (*μmol* NAD reduced/ *mg* protein/min.)	0.013±0.0067	0.026±0.0088	P<0.01
9.	17 β–HSD (*μmol* NADPH oxidized/ *mg* protein/min.)	0.0056±0.001	0.0074±0.001	P<0.02

Note : Mean±S.D., values are mean of six observations.

level of triglycerides was decreased with elevated lipase activity while the glycerol content was elevated, free fatty acid content was decreased. The levels of phospholipids and cholesterol of vasectomised animal testis were increased. The activity levels of 3 β-HSD and 17 β-HSD were significantly increased in the testis after vasectomy.

DISCUSSION

The testis of vasectomized rats after a prolonged peroid showed no significant change in tissue somatic index from control, which was suggestive of maintenance of fairly constant size of the testis after vasectomy. However, there was considerable increase in the water content which might be due to active uptake of water from the blood for hydrolytic activities, since the vasectomized testes recorded degeneration of the tissue (14, 23, 25). Since the dry matter was highly reduced, increased hydrolytic activities

resulting into overall degradation of organic components can be expected, which might reflect on the increased uptake of water. Accumulation of water by the tissues under atrophic conditions have been reported (11, 14, 22, 24) and hence water accumulation by the vasectomized testis was suggestive of active atrophic conditions in this tissue. Since carbohydrate and lipid fractions of the testis play vital role in the testicular activity, an attempt has been made to analyse some aspects of carbohydrate and lipid metabolism of tissue after vasectomy. The tissue glycogen content was highly elevated indicating the possibilities of increased glycogenesis and/or decreased glycogenolysis. Such an elevation in the glycogen content of vasectomized animal testis has been reported by previous workers (17). The values obtained in the present study were in perfect range of previous investigators. Lactic acid content of the testis of vasectomized rats was reported to be decreased (17), which was suggestive of decreased tissue glycolysis. However, sorbitol dehydrogenase activity was increased in the present study, which indicates active mobilisation of sorbitol into fructose and thereby increased fructolysis. The free glucose content was also considerably depleted. Under such conditions of inhibited glycolysis, mobilisation of hexoses into alternative pathways can be expected. The elevated activity of G-6-PDH was suggestive of mobilisation of glucose into hexose monophosphate pathway. Hence after vasectomy, there seems to be a shift in testicular carbohydrate metabolism from hexose diphosphate pathway to hexose monophosphate pathway. Since G-6-PDH activity is localised in leydig cells (9, 13), elevated enzyme activity suggests the activation of leydig cells after vasectomy. The previous observations indicating the proliferation of leydig cells of vasectomized animal testis (11, 20) were in support of such possibility. Such conditions result under active lipogenesis. Hence the testicular lipid fractions have been analysed for drawing correlations with changes in carbohydrate metabolism with testicular lipid profiles. The total lipid content was considerably elevated suggesting the possibility of increased lipogenesis. However, the triglyceride content was drastically decreased with an increase in lipase activity. This observation suggests the active mobilisation of neutral fat, into the testicular metabolism. Consequent on such an increase in the lipolysis, the glycerol content was elevated. But the free fatty acid content was depleted indicating the mobilisation of these components into oxidative metabolism. Hence after vasectomy the testicular metabolism seems to be oriented towards lipid oxidations resulting into decreased triglyceride content and elevated glycogen content. Such lipid utilisation property was associated with the testis under inhibited spermatogenesis (7). The phospholipid content was considerably elevated suggesting that the vasectomised animal testis was actively involved in the synthesis of phospholipid fractions. Since phospholipids were known to be associated with active transport mechanisms and biosynthetic activities (1) their increase in vasectomized rat testis was suggestive of the possibility of increased synthetic activities of the tissue with

promoted active transport processes. The cholesterol content of the tissue was drastically elevated suggesting stepped up steroidogenesis of the testis after vasectomy. This observation was in concurrence with the earlier reports (17, 25). Hence increased denovo synthesis of cholesterol in the testis can be expected. In order to understand the formation of androgens, key enzymes of the pathway have been analysed. 3 β-HSD activity, which marks the formation of progestogens was markedly increased suggesting mobilization of cholesterol into the steroid hormone formation. Similarly, the activity level of 17 β–HSD which marks the formation of testosterone was considerably elevated which was suggestive of increased androgenesis of the tissue. Since proliferation of leydig cells was witnessed in vasectomized animal testis (11, 20) and leydig cells were concerned with androgenesis, increased activities of 3 β-HSD and 17 β-HSD were indicative of stepped up androgenesis in the testis after vasectomy.

In general vasectomized rat testis showed a shift in metabolic mobilisation of organic compounds from carbohydrates to lipids, which might be responsible for inhibited spermatogenesis, reported by earlier investigators.

ACKNOWLEDGEMENTS

The authors (NG and KVR) are thankful to CSIR, New Delhi, for the award of fellowships during the tenure of which the present work is carried out.

REFERENCES

1. Anfinsen, C.B. Jr. Chapter 7. In Symposium on Atherosclerosis. Discussion National Research Council, Washington D.C., pp. 167–179, 1954.
2. Bergmeyer, H.U. and E. Bruns. Methods of Enzymatic Analysis. (ed) H.U. Bergmeyer, Academic Press, New York, 1965.
3. Bergmeyer, H.U. Methods of enzymatic analysis. (ed). H.U. Bergmeyer, Verlag Chemie Weinheim, Academic Press, Inc., New York, Vol. 1, 1974.
4. Bieri, J.G. and E.L. Prival. Lipid composition of testis of various species. *Comp. Biochem. Phisiol.*, **15** : 271–281, 1965.
5. Burton, R.N. The determination of glycerol and dihydroxy acetone as methods in enzymology, Vol. III (eds), S.P. Colowick and N.O. Kaplan. Academic Press, Inc. Publishers, New York, pp. 246–281, 1957.
6. Chatterjee, S.N., R.N. Menakshi Sharma, V.P. Kamboj and B. Amiya Kar. Proceedings of the Seminar on Fertility control held under the auspices of Sri Venkateshwara University, Tirupati, India, pp. 130, 1976.
7. Fleeger, J.L., J.P. Bishop, W.R. Gomes and N.L. Vandemark. Effect of unilateral cryptorchidism on fatty acids of testicular phospholipids and triglycerides. *J. Reprod. Fertil.*, **15** : 9–14, 1968.
8. Folch, J.M., P. Lees and G.H. Stane-Stanley. A simplified method of isolation and purification of total lipids from animal tissue. *J. Biol. Chem.*, **226** : 497-501, 1957.
9. Gaitonde, S.G., S.K. Saidapur and B.Y.M. Gowder. The sites of steroidsynthesis in the testis of Hawk Accipiter badius (Gmelin) : A Histochemical Study. *Curr. Sci.*, **50** : 915–916, 1981.

10. Huggins C. and J. Lapides. Lipase : Method in Enzymol (eds) S.P. Colowick and N.O. Kaplan Academic Press. New York, **1** : 627–642, 1955.

11. Jhaver, P.S. and B.B. Ohri. The History of experimental and clinical work on vasectomy. *J. Int. Coll. Surg.*, **33** : 482, 1960.

12. Kemp, A. and M.K. Van Heijningen, A colorimetric micromethod for the determination of glycogen in tissue. *Biochem. J.*, **56** : 646–651, 1954.

13. Knorr, D.W., T. Vanha-Perttula and M.B. Lipsett. Structure and function of rat testis through pubescence. *Endrocrinol.*, **86** : 1298–1304, 1970.

14. Lohiya N.K. As cited in abstract IV All India Symposium on Comparative Animal Physiology and Endocrinology, Sri Venkateswara University, Tirupati, India, Dec., pp. 90, 1978.

15. Mendel, B., A. Kemp. and D.K. Myers. A colorimetric micromethod for the determination of glucose. *Biochem. J.*, **56** : 639–645, 1954.

16. Meenakshi, S.N. Chetterjee, R.N. Sharma, V.P. Kamboj, and A.B. Kar. Effect of normal defective vasectomy on the urinary 17-ketosteroid excretion rate and total pituitary gonadotrophin content in dog. *Indian J. Exp. Biol.*, **14** : 95–96, 1976.

17. Meenakshi, S.N. Chetterjee, R.N. Sharma, V.P. Kamboj, and A.B. Kar. Effect of normal and defective vasectomy on the biochemistry of testis and accessory genital organs of dogs. *Indian J. Exp. Biol.*, **14** : 641–646, 1976.

18. Nachlas, M.M., S.P. Morgulis and A.M. Seligmen. A colorimetric method for estimation of succinic dehydrogenase activity. *J. Biol. Chem.*, **235** : 449–504, 1960a.

19. Natelson, S. Techniques of clinical chemistry (3rd Edn), Charles. C Thomas Publishers, Illinois, U.S.A., 1971.

20. Neaves, W.B. The androgen status of vasectomized rats. *Endocrinol.*, **96** : 529–534, 1975.

21. Phadke, G.M. In vasectomy (Technique), Directorate General of Health Services, Ministry of Health, Government of India, New Delhi, 1962.

22. Rangam, C.M., M.M. Arora and J.G. Gupta. Testicular changes in rats following vasectomy. *Indian J. Med. Sci.*, **11** : 717, 1957.

23. Sackler, A.M., A.S, Weltman, V. Pandhi and K. Schwartz. Gonadal effect of vasectomy and vasoligation. *Science*, **179** : 293, 1973.

24. Thakur, A.N., A.R. Sheth and S.S. Rao. Biochemical studies on rat testis and sex accessory organs after vasoligation operation. *Fert. Steril.*, **23** : 834, 1972.

25. Vemananda Reddy, G., P. Reddanna and S. Govindappa. Effect of bilateral vasectomy on tissue cholesterol distribution in albino rats. *Curr. Sci.*, **49** : 179–182, 1980.