EFFECT OF ESTRADIOL-17β AND PGF$_2\alpha$ ON GLYCOGEN METABOLISM OF THE ALBINO RAT TESTIS

C. CHANGAMMA and P. REDDANNA*
Regional Research Laboratory, Jammu 180001, India
**Department of Zoology, S. V. University, Tirupati 517502, India.*

ABSTRACT

Wistar strain adult albino rats were administered subcutaneously by estradiol-17β and PGF$_2\alpha$ for seven days. The glycogen metabolism of the testis was studied. The decreased glycogen content was witnessed through stepped-up glycogenolysis. Inspite of elevated glycogenolysis, the testis glucose was decreased, which might reveal its possible mobilization towards either hexose monophosphate or diphosphate pathways. The elevated levels of phosphorylase *a*, *b* and *ab* by these treatments suggested an over all increment in the concentration of enzyme itself. The elevated FDP-aldolase by the estradiol-17β treatment suggested the stepped-up operation of hexose diphosphate pathways. The increased content of lactic acid in estradiol-17β treated rat testis suggest the mobilization towards the pyruvic acid. On the other hand the decreased pyruvic acid by the PGF$_2\alpha$ treatment suggests the possible stepped-up mobilization of lactic acid towards the pyruvic acid.

INTRODUCTION

AMONG the earliest and most widespread efforts directed at controlling male fertility were studies on those compounds which specifically or broadly affected the spermatogenic process without directly altering pituitary control mechanisms[1]. Some such compounds are alkylating, estrogenic and progestogenic. The estrogenic compound like estradiol-17β treatment induced dose-related reduction of sperm population and eventual azoo-spermia, atrophy of accessory organs and suppressed serum LH and testosterone levels[2]. At low levels, estradiol-17β exhibits positive feedback properties on serum FSH in male rats[3].

The other antifertility agent is prostaglandin F$_2\alpha$. It is a luteolytic and it is used in several species to control fertility. It causes degeneration of spermatocytes and decreases the number of spermatids[4]. Subcutaneous injections of PGF$_2\alpha$ to male rats promoted definite growth and had partial antifertility effects[5].

Further, most of the studies on estradiol and PGF$_2\alpha$ treatments have been confined to only histological, histo-chemical and hormonal assay studies. Very little information is available towards the effect of estradiol on the testicular energy metabolism. A constant supply of glucose is considered to be an essential requirement for the proper functioning of the testis[6]. In view of this, the glycogen metabolism of the testes was undertaken for investigation in the present study.

MATERIALS AND METHODS

Healthy adult male Wistar strain albino rats of 90 days age group weighing 180 $\pm$ 10 g were selected for experimentation. The rats were maintained at the laboratory conditions (27° $\pm$ 2°C; 12 hr light exposure and 12 hr darkness). The animals were fed on standard rat feed obtained from Hindustan Lever Ltd., Bombay India and water was supplied *ad libitum*.

The animals were divided into two groups: control and experimental. The experimental group of rats were divided into two batches of six each. One batch of rats received subcutaneous injections of estradiol-17 β (Sigma Chemical Co., USA) at the dose of 1 mg/kg body weight/day dissolved in 0.5 ml of sesame oil for 7 days. Second batch of rats received subcutaneous injections of prostaglandin F$_2\alpha$ (UP John Company, Calamazoa, Michigan, USA) dissolved in 95% ethanol to which physiological saline (0.9%) was added to get the required dilution at the dose of 1 mg/kg body weight/day for 7 days.

The control group of rats received similar dose of sesame oil and physiological saline (0.9%NaCl). The control and experimental group of rats were sacrificed by cervical dislocation 24 hr after the last dose of treatment and the testes were isolated, chilled immediately and used for biochemical analysis. The glucose[7], glycogen[8], lactic acid[9], pyruvic acid[10], the activity levels of phosphorylase[11] and aldolase[12] were estimated in control and experimental rat testes. Student *t* test is used to analyse the data.

RESULTS

Tables 1 and 2 shows that both estradiol-17β and prostaglandin $F_2\alpha$ administration induced significant changes in testes glycogen metabolism. The glycogen and glucose content of testes were decreased significantly by the administration of these agents when compared to that of control rat testes, the decrease being more in glycogen content in both of the administrations. Pyruvic acid content showed non-significant change while the lactic acid on the other hand showed increment (20%) in estradiol-17β treated rat testes. On the contrary the lactic acid showed non-significant change while the pyruvic acid showed decreased tendency (87%) in the $PGF_2\alpha$ treated rat testes.

The glycogen phosphorylase *a* activity level was elevated significantly by both of the administrations, the elevation being more in the case of $PGF_2\alpha$ treated rat testes. The total and inactive form of phosphorylase also elevated in treated rat testes when compared to that of control rat testes.

Table 1 *The levels of glycogen, glucose, lactic acid, pyruvic acid and phosphorylase*

Parameters	Control	Percent change	Estradiol-17β treated
Glycogen (mg/g fresh tissue)	1.20 ±0.16	−50.75*	0.591 ±0.075
Glucose (mg/g fresh tissue)	0.813 ±0.15	−28.66*	0.580 ±0.077
Lactic acid (mg/g fresh tissue)	8.21 ±0.53	+20.46**	9.89 ±0.88
Pyruvic acid (μmol/g fresh tissue)	37.95 ±1.85	−6.19***	35.62 ±1.90
Phosphorylase-*a* (μmol pi formed/mg protein/hr)	0.412 ±0.038	+99.03*	0.820 ±0.056
Phosphorylase-*ab* (μmol pi formed/mg protein/hr)	0.889 ±0.035	+66.93*	1.484 ±0.089
Phosphorylase-*b* (μmol pi formed/mg protein/hr)	0.477 ±0.055	+40.67*	0.671 ±0.08
Aldolase (μmol FDP cleaved/ mg protein/hr)	3.509 ±0.113	−9.09**	3.190 ±0.138

* = $P < 0.001$; ** = $P < 0.01$; *** = NS

a, *b* & *ab* and aldolase in testis of control and estradiol-17β treated rats. Mean ± S.D. of six individual observations. + and − indicate percent increase and decrease respectively over control. *p* denotes the level of significance and NS non-significance.

Table 2 *The levels of glycogen, glucose, lactic acid, pyruvic acid and phosphorylase*

Parameters	Control	Percent change $PGF_2\alpha$	treated
Glycogen (mg/g fresh tissue)	1.39 ±0.12	−57.19*	0.595 ±0.032
Glucose (mg/g fresh tissue)	0.653 ±0.121	−37.67*	0.407 ±0.044
Lactic acid (mg/g fresh tissue)	8.68 ±0.87	−2.30***	8.48 ±0.71
Pyruvic acid (μmol/g fresh tissue)	36.88 ±1.37	−87.5*	4.61 ±0.73
(phosphorylase *a* (μmol pi formed/mg prot/hr)	0.391 ±0.018	+199.74*	1.172 ±0.056
Phosphorylase *ab* (μmol pi formed/mg prot/hr)	0.857 ±0.042	+137.92*	2.039 ±0.086
Phosphorylase *b* (μmol pi formed/mg prot/hr)	0.466 ±0.042	+84.55*	0.860 ±0.090
Aldolase (μmol GDP cleaved/mg)	3.512 ±0.200	+104.47*	7.181 ±0.250

* = $P < 0.001$; *** = NS

a, *b*, & *ab* and aldolase in testis of control and $PGF_2\alpha$ treated rats. Mean ± S.D. of six individual observations. + and − indicate percent increase and decrease respectively over control. *P* denotes the level of significance and *** non-significance.

The activity level of FDP-aldolase was slightly decreased ($P < 0.01$) in the estradiol-17β treated rat testis while it is highly elevated in $PGF_2\alpha$ treated rat testis.

DISCUSSION

The administration of estradiol-17β and $PGF_2\alpha$ induced significant changes in testes glycogen metabolism. Generally the reproductive tissue like testes, largely depends on the carbohydrates for the spermatogenesis[13, 14]. Hence, the study of carbohydrate metabolism was undertaken to understand any impairment in the carbohydrate metabolism.

It is reported that the glycogen reserves were localized in the spermatogonia and spermatocytes of

the testes[14]. The glycogen concentration was almost halved in testes of estradiol-17β and $PGF_2\alpha$ treated rats showing the possibility of increased glycogenolysis or decreased glycogenesis. The glycogenolysis was assessed by estimating the phosphorylase *a* activity. The activity of phosphorylase *a* showed a two-fold elevation suggesting the increased rate of mobilization of glycogen in estradiol-17β treated rat testes. The reported increase in the cyclic AMP levels in the tubules of $PGF_2\alpha$ treated rats[15] might be responsible for the highly elevated phosphorylase *a* activity observed in the present study. Besides the elevated phosphorylase *a* activity the inactive and total forms of phosphorylase were also elevated suggesting the possible *de novo* synthesis of the enzyme. The increased phosphorylase *ab* supports such a possibility. Inspite of elevated glycogenolysis, glucose concentration was significantly decreased, which might be due to increased mobilization of glucose into the Embden Mayer hof and hexose monophosphate pathways. The significantly elevated glucose-6-phosphate dehydrogenase activity level suggested the possible mobilization of glucose into hexose mono-phosphate pathway. Besides this, the decreased blood flow to the testis in $PGF_2\alpha$ treated rats[16] might also be responsible for the decreased glucose reserves. The depleted aldolase activity also indicates decreased mobilization of glucose into Embden Mayerhof pathway.

The pyruvic acid concentration showed non-significant change whereas the lactic acid was increased significantly in estradiol-17β treated rat testis. The increased lactic acid content inspite of elevated activity level of NAD-LDH suggests the possible formation of pyruvate from some source other than glycolysis. In the case of $PGF_2\alpha$ treatment, the pyruvic acid concentration was highly depleted with no change in the lactate concentration. The decreased pyruvate and no change in the lactate inspite of highly elevated glycogenolysis and glycogenesis suggest the higher shunting of pyruvate into the citric acid cycle. The elevated NAD-LDH indicates such a possibility.

Thus, the glycogen metabolism in the estradiol-17β and $PGF_2\alpha$ treated rats seems to be oriented towards decreased glycogen and glucose concentrations through the activation of glycogenolysis and hexose mono phosphate pathways. The decreased energy reserves might be responsible for the reported reduction in sperm population and degeneration of Leydig cells[3]. The decreased glycogenolysis and glycolysis resulting into decreased energy production might be responsible for reduction in sperm population[2] spermatocytes and spermatogonial population[17].

$PGF_2\alpha$ enhanced testicular glycogenolysis and glycogenesis and decreased and glycogen and glucose concentrations. These biochemical changes might probably be reflecting in the impaired spermatogenesis, as well as reduction and degeneration of spermatocytes. The impaired spermatogenesis, in spite of enhanced glycogenolysis might be due to reported decrease in androgen production and suppressed testosterone levels[18].

ACKNOWLEDGEMENTS

CC is grateful to CSIR for a fellowship.

31 December 1984

1. Gomes, W. R., Pharmacological agents and male fertility, In: *The Testis*, vol. II, (eds) A. D. Johnson, W. R. Gomes and N. L. Van Demark, Academic Press, New York, London, 1977, p. 605.
2. Hunt, M. O., Saksena, S. K. and Chang, M. C., *Archives of Andrology*, 1979, **21**, 129.
3. Kanamadi, R. D. and Saidapur, S. K., *Indian J. Exp. Biol.*, 1982, **20**, 209.
4. Chinoy, N. J. and Chinoy, M. R., *Int. J. Fertil.*, 1981, **26**, 1.
5. Chinoy, N. R., Sharma, J. D., Seethalakshmi, L. and Sanjeevan, A. G., *Int. J. Fertil.*, 1980, **25**, 267.
6. Free, M. J., Carbohydrate metabolism in the testis. In: *The Testis*, vol. II (eds) A. D. Johnson, W. R. Gomes and N. L. Van Demark, Academic Press, London, 1970, p. 125.
7. Mendal, B., Kemp, A. and Myers, D. K., *Biochem. J.*, 1954, **56**, 639.
8. Kemp, A. and Van Heijningen, M. K., *Biochem. J.*, 1954, **56**, 646.
9. Huckabee, W. E., *Am. J. Med.*, 1961, **30**, 833.
10. Freideman, T. E. and Hangen, G. E., *J. Biol. Chem.*, 1942, **147**, 67.
11. Cori, G. T., Illingworth, B. and Killer, P. G., Muscle Phosphorylase, In: *Methods in Enzymology* (eds) S. P. Colowick and N. O. Kaplan. Academic Press, New York, 1955, p. 200.
12. Brums, F. H. and Bergmeyer, H. . Fructose-1, 6-diphosphate aldolase. In: *Methods of enzymatic analysis* (ed.) H. O. Bergmeyer, Academic Press, New York, 1965, p. 724.
13. Ewing, L. L., Means, A. R., Beams, C. G., Montgomery, J. R. and Montgomery, R. D., *J. Reprod. Fertil.*, 1966, **12**, 295.

14. Leiderman, B. and Mancini, R. E., *Endocrinology*, 1969, **95**, 607.
15. Cho-chung, Y. S. and Gullio, P. M., *Science*, 1974, **183**, 87.
16. Free, M. J. and Jaffe, R. A., *Prostaglandins*, 1972, 483.
17. Elkington, J. S. H. and Blakshaw, A. W., *Aust. J. Biol. Sci.*, 1971, **24**, 1263.
18. Edwards, S. F., John, R. Diehl, Richard Barb, C., Terry, E. Keiser, Robert, R., Kraeling and George, B., *Prostaglandins*, 1981, **21**, 933.

NEWS

NEW SODIUM-FREE SALT SUBSTITUTE

. . . "First came aspartame, a simple compound with the taste of sugar but without the calories. Now there is ornithyltaurine, with the taste of salt but without the sodium. Makato Tada, Ichizo Shinoda, and Hideo Okai of Hiroshima U, recently synthesized ornithyltaurine and three other compounds that mimic the taste of salt. Since none of the new chemicals contains sodium, their discovery is good news for the estimated 13% of Americans whose doctors have put them on low or no-sodium diets because of high blood pressure or other disorders. Moreover, say the chemists, the impostors lack the bitter after-taste of the currently available salt substitute, potassium chloride. . . . The researchers say that ornithyltaurine, the saltiest of the synthetic peptides found so far, is twice as salty as the real thing; most of the new monosodium glutamate-like compounds are about as strong as their natural counterparts."

[(In *Science 85* 6(2):8, 12, Mar 85). Reproduced with permission from Press Digest, *Current Contents*®, No. 16, April 22, 1985, p. 12 (Published by the Institute for Scientific Information®, Philadelphia, PA, USA.)]

GRAVITY WAVES AND SPACEQUAKES

. . . "Einstein's equations revealed that if a mass were suddenly accelerated or jostled to and fro, it would generate ripples in that sheet of space-time, similar to the way electrons moving along an antenna generate radio waves in the air. But while such electromagnetic waves travel *through* space, gravity waves actually disturb the fabric of space. This space-time rippling occurs every time you bang your fist on a table or jump rope, but only the most awesome cosmic events emit any appreciable waves. Particles and planets caught in the path of such a wave would experience space itself contracting and expanding. Such a 'spacequake' would provide astronomers with an entirely new form of information and the universe. 'Visible and infrared light, radio waves, and X-rays are emitted almost entirely by individual atoms, molecules, and high-energy particles,' explains Kip Thorne [Caltech]. 'Gravitational waves, by contrast, are emitted by the bulk motions of huge amounts of matter, objects that are vibrating, collapsing, or exploding.' More important, these periodic distortions in the structure of space-time can blithely pass through interstellar dust, planets, and galaxies as if they weren't there. Nothing can be absorb them. This penetrating power may allow astrophysicists to observe cosmic processes that, for now, can only be imagined on a computer graphics terminal—from the last millisecond gasp in the life of a star to the titanic nllision of two black holes."

[(Marcia Bartusiak in *Science 85* 6(3): 58-65, Apr 85. Reproduced with permission from Press Digest, *Current Contents*®, No. 18, May 6, 1985, p. 16, (Published by the Institute for Scientific Information®, Philadelphia, PA, USA.)]