Effect of prostaglandins A-1, E-2 and F-2 α on spermatogenesis in rats

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Summary. Rats were treated with twice daily injections of 100 μ g PG/rat for 15 days. PGA-1 had no effect. PGE-2 caused a significant increase in testicular weight, RNA content, hyaluronidase activity and number of spermatids. PGF-2 α produced a significant decrease in sorbitol dehydrogenase activity and DNA content. It is suggested that PGE-2 may be involved in later stages of spermatogenesis, i.e. conversion of spermatocytes to spermatids.

Introduction

Saksena, El Safoury & Bartke (1973), Saksena, Lau & Bartke (1974) and Bartke, Musto, Caldwell & Behrman (1973) have shown that there is a decrease in plasma testosterone concentrations after subcutaneous administration of prostaglandin (PG) E-2 or F-2 α in rats and mice respectively. The decrease was considered to be due to decreased plasma levels of LH. We observed histologically suppression of spermatogenesis in rats after inhibition of PG synthesis by aspirin treatment for 30 days (unpublished observation). When indomethacin was administered to male rats for 30 days no change in FSH concentrations was observed (Saksena, Lau, Bartke & Chang, 1975). Biswas, Sanyal & Patra (1978) have reported a reversion of aspirin-induced impairment of spermatogenesis by treatment with PGE-2. The precise role of PGs in spermatogenesis has not yet been elucidated and the present investigation of the activities of various marker enzymes of spermatogenesis was therefore undertaken.

Materials and Methods

Animals. The 28 male albino rats weighed 250–300 g and were of proven fertility. They were kept in controlled laboratory conditions (14 h light/24 h and $28 \pm 1^\circ\text{C}$). Laboratory diet and water were always available. The rats were equally allocated to 4 groups which received twice daily (06:00 h and 18:00 h) for 15 days intraperitoneal injections of 100 μ g PGA-1 (Group 2), PGE-2 (Group 3) or PGF-2 α (Group 4) made up in sterile saline (9 g NaCl/l), or 0.5 ml sterile saline (Group 1).

The prostaglandins were obtained from Dr J. E. Pike, Upjohn Co., U.S.A., as a gift. A solution of PG was made up in ethanol (5 mg/ml) and stored in a refrigerator. The desired dilutions were made from these ethanolic solutions with sterile saline. Solutions for injections were made freshly before injection.

The rats were decapitated 3–4 h after the last injection. The testes were immediately excised, blotted and weighed. Part of one testis was kept for histological studies and part stored at -10°C until further assays. To minimize the error due to loss of enzyme activity during storage the experiment was planned so that Groups 3 and 4 were killed on one day and Groups 1 and 2 on the next. Identical storage conditions were maintained for all the groups. Assays of β -

glucuronidase and hyaluronidase were carried out on the day of killing and sorbitol dehydrogenase activity was measured on the 4th day. The temperature was maintained at 0–4°C throughout the extraction procedure. The two bits of testes were assayed individually for DNA and RNA estimations. All the enzyme estimations are carried out in duplicate.

Assays. Testicular homogenate was prepared for enzyme assays by the method of Males & Turkington (1971), β -glucuronidase was assayed by the method of Fishman (1967) and hyaluronidase by method of Males & Turkington (1970). Homogenate for sorbitol dehydrogenase assay was made after thawing the tissue and activity was measured by the spectrophotometric method of King & Mann (1966).

Testicular nucleic acids were extracted by the method of Munro & Fleck (1966), and RNA was estimated by the orcinol method (Ceriotti 1955) and DNA by the method of Burton (1956). Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

Histology. A part of the right testis of each rat was taken for histological studies. Testes were fixed in Bouin's fluid and 5 μ m paraffin-wax sections were stained with haematoxylin and eosin. The quantitative analysis of the seminiferous epithelium was performed on the basis of the relative number of germ cell nuclei/section of seminiferous tubule at stage VII of the cycle (Clermont & Morgentaler, 1955). For counting the germ cell number, 4 or 5 round cross-sections of tubule per rat were used, i.e. a total of 20 cross-sections in each group. The nuclear counts were corrected for differences in nuclear diameter by the formula of Abercrombie (1946).

Results

A significant increase in the testicular weight was seen only in Group 3 (Table 1). DNA content was affected only in Group 4 but there was a significant increase of RNA in all 3 groups of PG-treated rats. Table 2 shows the effects of PGs on the enzyme activities studied. Compared with

Table 1. Effects of prostaglandins on mean $\pm$ s.e.m. testicular weight and DNA and RNA content in rats (7/group)

Group	Body wt (g)	Testes wt (g/100 g body wt)	DNA (mg/g wet wt)	RNA (mg/g wet wt)
1 (controls)	322.14 $\pm$ 9.25	0.89 $\pm$ 0.05	1.538 $\pm$ 0.054	2.122 $\pm$ 0.035
2 (PGA-1)	293.42 $\pm$ 7.49	0.86 $\pm$ 0.11	1.619 $\pm$ 0.078	2.52 $\pm$ 0.09**
3 (PGE-2)	276.42 $\pm$ 9.24	1.01 $\pm$ 0.038*	1.774 $\pm$ 0.13	3.118 $\pm$ 0.082**
4 (PGF-2 α)	277.85 $\pm$ 9.19	0.96 $\pm$ 0.031	1.298 $\pm$ 0.022**	3.452 $\pm$ 0.10**

Significantly different from control value: * P < 0.05; ** P < 0.001.

Table 2. Effect of prostaglandins on mean $\pm$ s.e.m. activity of some testicular enzymes in rats (7/group)

Group	β -Glucuronidase†	Sorbitol dehydrogenase‡	Hyaluronidase§
1 (controls)	6.40 $\pm$ 0.80	7.38 $\pm$ 0.26	29.54 $\pm$ 2.39
2 (PGA-1)	10.76 $\pm$ 2.35	6.94 $\pm$ 0.70	39.96 $\pm$ 6.85
3 (PGE-2)	8.62 $\pm$ 4.45	10.18 $\pm$ 2.2	49.85 $\pm$ 2.43*
4 (PGF-2 α)	7.37 $\pm$ 0.82	3.37 $\pm$ 0.88*	25.12 $\pm$ 4.18

* Values significantly different from corresponding control, P < 0.001.

† Measured as μ g phenolphthalein liberated/mg protein.

‡ Measured as the change in optical density of 0.001/min/mg protein.

§ Measured as amount of hexosamine liberated in μ /mg protein.

Group 1, sorbitol dehydrogenase values differed significantly only in Group 4, while hyaluronidase values differed only in Group 3. The only effect on the seminiferous epithelium which could be observed was the increased number of spermatids in Group 3 rats (Table 3). No morphological changes could be seen in any of the cells.

Table 3. Analysis of cell numbers (mean $\pm$ s.e.m., 20 round cross-sections/group) in seminiferous tubules of prostaglandin-treated rats

Group	No. of rats	'A' type spermatogonia	Resting spermatocytes	Pachytene spermatocytes	Spermatids (stage 7)	% Degeneration of spermatids
1 (controls)	5	0.77 $\pm$ 0.21	18.22 $\pm$ 1.67	21.26 $\pm$ 2.7	76.74 $\pm$ 3.6	10
2 (PGE-2)	4	0.81 $\pm$ 0.16	17.61 $\pm$ 1.8	24.54 $\pm$ 2.66	94.53 $\pm$ 5.2*	2.5
3 (PGF-2 α)	5	0.74 $\pm$ 0.26	19.0 $\pm$ 1.6	20.57 $\pm$ 1.27	66.38 $\pm$ 4.1	20

* Significantly different from corresponding control, $P < 0.05$.

Discussion

The increased testicular weight in rats treated with PGE-2 was presumably due to the increased number of cells in the seminiferous epithelium.

Males & Turkington (1971) observed that β -glucuronidase activity is associated with the spermatogonia or Sertoli cells and that the level of this enzyme decreases during senescence. Any change in the number or function of spermatogonia or Sertoli cells would therefore be reflected in the activity of testicular β -glucuronidase. We found no change in the activity of this enzyme in any of the treated animals, suggesting that the spermatogonia and Sertoli cells were functioning normally. This was confirmed by the unchanged numbers of A-type spermatogonia.

Panko, Noller & Howards (1977) have suggested that levels of DNA and RNA may act as indices of the process of spermatogenesis, especially the later stages. In the present investigation DNA and RNA concentrations were increased in the rats treated with PGE-2 and this could indicate stimulation of later stages of spermatogenesis.

Sorbitol dehydrogenase activity parallels the appearance of advanced germ cells; Bishop, Scharnk, Musselman & Muecke (1967), Bishop, Tang, Walker & Kenner (1970) and Mills & Means (1972) have found a correlation between the activity of this enzyme and meiosis and later stages of spermatogenesis. Hyaluronidase activity is particularly associated with spermatids in the testis (Steinberger & Nelson, 1955; Males & Turkington, 1970), the most marked decrease in enzyme levels occurring when spermatid formation and metamorphosis is impaired.

Our results show an increase in the number of spermatids after PGE-2 treatment, which was due to prevention of degeneration of the spermatids. The theoretical 1:4 ratio of pachytene spermatocytes to spermatids (Clermont & Morgentaler, 1955) was nearly achieved in the PGE-2 treated rats (1:3.9 compared with 1:3.6 in the controls).

These results run counter to those obtained for mice by Abbatiello, Kaminsky & Weisbroth (1975, 1976) who reported a decrease in the number of spermatocytes and spermatids after administration of PGE-2 and an increase after administration of PG inhibitors such as aspirin and indomethacin. Biswas *et al.* (1978) found that aspirin treatment of adult male rats led to a decrease in the number of spermatids, which could be remedied by PGE-2 administration. Our data are in agreement with those of Biswas *et al.* (1978) and the discrepancy with the results of Abbatiello *et al.* (1975, 1976) may be ascribed to the use of different species and higher dose of PG.

The activities of sorbitol dehydrogenase and hyaluronidase are regulated by gonadotrophins, particularly FSH. Levels of these enzymes can be maintained by FSH alone in hypo-

physectomized rats (Males & Turkington, 1970; Mills & Means, 1972). The formation of spermatids from spermatocytes is also regulated by FSH (Steinberger, 1971). Our data therefore suggest that the enhancement of later stages of spermatogenesis after PGE-2 treatment may be mediated through FSH.

We conclude that PGE-2 may be involved in the regulation and maintenance of spermatogenesis, perhaps by regulating the conversion of secondary spermatocytes to spermatids and affecting FSH secretion. The effects of PGF-2 α seemed to be contrary to those of PGE-2 while PGA-1 seemed to have no effects.

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