HORMONAL INFLUENCE ON EPIDIDYMAL LIPIDS

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Summary: Single dose administration of Prolactin(P), Progesterone (PP) and a combination of both (PPP) affected the epididymal lipids considerably. Caput and Cauda showed differential responses. PP and PPP showed significant alterations in Caput epididymis. However, Prolactin was effective in Cauda epididymis. The importance of these changes in relation to physiological functions of epididymis is discussed.

Key words : prolactin progesterone epididymis

INTRODUCTION

The present approach to male contraception is aimed mainly to alter a slight but specific biochemical or physiological environment in the accessory sex organs, to render the sperm non-viable. Epididymis proves to be the best site for such an attempt as the sperms have to undergo a short sojourn in the epididymis for their maturation to effect movement and fertilization (16). Studies relating to the functions, hormonal effects and biochemical composition of epididymis have been already reported (17). Epididymal lipids have mainly been found to fluctuate during maturation processes of sperm (17,18). Progesterone has been reported to affect sperm maturation in hamsters (12). Both prolactin and progesterone were implicated in the altered testicular lipid pattern accompanying degenerative changes in testicular tissue. The inhibitory response of epididymal tissue lipids to antiandrogens have already been reported from this laboratory (9). In the present investigations, an attempt has been made to study the short term effect of these hormones on epididymal lipids.

MATERIALS AND METHODS

Animals :

100-day-old, male albino rats of Wistar strain (190-200 g) were taken for the present studies. They were divided into four groups, each consisting of 10 animals. The first group received progesterone (BDH, Poole, England) 1 *mg*/100 *g* body weight/day intramuscularly. The second group received prolactin (Bovine Prolactin NIH-P-B_3) 1 *mg*/100 *g* body weight/day. The third group received progesterone plus prolactin (1 *mg*/100 *g* body weight each). The fourth group served as control and received only the vehicle

(pea nut oil). 24 hours after administration of the respective hormones, the animals were sacrificed by cervical dislocation. The caput and cauda epididymides were rapidly dissected, freed from adhering connective tissues, rinsed, blotted and weighed accurately on a torsion balance.

The lipids were extracted from the tissues repeatedly by a mixture of chloroform: methanol (2:1, V/V) according to the procedure of Folch *et al.* (7). The total lipids were determined gravimetrically (7). Total phospholipids were determined as phospholipid phosphorus. The inorganic phosphorus was determined by the method of Bartlett as modified by Marinette (14). The values of inorganic phosphorus was multiplied by a factor 25 to get the phospholipid values (4). The method of Tschugaeff as modified by Hanel and Dam (11) was used to estimate total cholesterol.

Separation and identification of neutral lipids :

The neutral lipids were isolated by applying an aliquot of total lipids on silica gel G coated plates (20x20cm, 250 nm thickness). They were run in the first solvent system (n.hexane: diethyl ether: glacial acetic acid, 60:40:1 by volume) upto a height of 7 cm, dried and run in the second solvent system (n.hexane: diethyl ether : glacial acetic acid, 90:10:1 by volume) to a height of 15 cm. Authentic standards (Tristearin, Monostearin, Cholesterol stearate and Cholesterol, BDH, Poole, England) were run along with the samlpes and were identified in an iodine chamber. The appropriate fractions were scrapped out, extracted with cholroform and processed for the determination of cholesterol and glyceride glycerol respectively.

Separation and identification of phospholipids :

An aliquot of total lipids containing about 10-12 μg of lipid phosphorus was applied on thin layer chromatography plates coated with silica gel G (20 x 20 cm, 250 nm thickness) for the separation of phospholipid classes. They were run in the solvent system, chloroform: methanol : ammonia (7N) (115:45:7.5 by volume (1). The fractionated phospholipids were identified with the use of authentic standards (Phosphatidyl serine, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, sphingomyelin, P.L. Biochemicals, U.S.A.) with molybdenum blue spray (6).

The level of significance between treatment groups were calculated using Student 't' test.

RESULTS

Caput epididymis :

Table I depicts changes in lipid composition of caput under the influence of P, PP and PPP. PP affected an increase in free, esterified cholesterols as well as in mono-and triglycerides ($P<0.005$). PPP brought about an elevation in free, esterified cholesterols and in di- and triglycerides ($P<0.001$).

TABLE I : Changes in total lipid and neutral lipid fractions of caput epididymis (expressed as *mg/gm* tissue single day treatment.

Parameters	*Control*	*Prolactin*	*Progesterone*	*Prolactin and progesterone*
Total lipids	105.0±20.2	108.3±19.2	***140.8±22.0	*200.3±30.8
Neutral lipids	85.0±14.3	90.3±18.7	118.5±24.1	*167.5±34.5
Phospholipids	20.0± 2.0	18.0± 2.2	22.3± 1.8	**32.8± 2.2
Cholesterol	11.0± 1.2	10.8± 1.3	**20.8± 1.7	*30.2± 2.8
Glycerides	74.0±12.3	79.5±14.2	***97.7±15.3	*137.3±20.2
Free cholesterol	1.7± 0.2	1.8± 0.3	** 2.3± 0.8	**3.2± 0.8
Mono-glycerides	30.8± 5.2	**18.5± 2.2	**17.5± 2.3	22.4± 3.0
Diglycerides	29.3± 3.8	40.7± 3.8	41.0± 3.2	*56.0± 5.2
Triglycerides	14.4± 1.2	20.3± 2.0	**39.2± 2.8	*58.9± 5.8

Values are mean ±S.D. of 10 animals/group.

* $P<0.001$

** $P<0.005$

*** $P<0.05$

Table II depicts changes in phospholipid fractions of caput under the influence of P, PP and PPP. PPP alone favoured the accumulation of all fractions of phospholipids ($P<0.005$) with a subsequent decrease in the precursor, phosphatidic acid.

TABLE II : Changes in the fractions of phospholipids in caput epididymis (expressed as *mg/g* tissue).

Parameters	*Control*	*Prolactin*	*Progesterone*	*Prolactin and progesterone*
Phosphatidyl inositol and phosphatidyl serine	1 9±0.6	1.3±0.6	2.1±0.3	**4.2±0.9
Lysophosphatidyl choline	2.9±0.8	2.8±0.9	3.0±0.2	**4.8±0.8
Lysophosphatidyl ethanolamine and sphingomyelin	2.1±0.7	3.0±1.0	3.0±0.3	**5.2±1.2
Phosphatidyl choline	5.2±1.2	5.1±1.1	6.3±1.3	**9.8±1.0
Phosphatidyl ethanolamine	3.9±0.8	2.8±0.8	4.2±0.9	**6.3±0.8
Phosphatidyl glyceryl phosphate and phosphatidic acid	4.0±1.1	3.0±0.8	2.7±0.8	2.5±0.7

Values are mean ±S.D. of 10 animals/group.

** $P<0.005$

** $P<0.05$

Cauda epididymis :

Table III shows changes in lipid profile of cauda due to the administration of P, PP and PPP. Prolactin brought about a decrease in total lipids ($P<0.005$) due to a fall in mono-, di- and triglycerides ($P<0.005$). PP and PPP caused an accumulation in free and esterified cholesterols ($P<0.005$).

Table IV shows the changes in the fractions of phospholipids in cauda epididymis.

TABLE III : Changes in total lipid and neutral lipid fractions of cauda epididymis (expressed as *mg/g* tissue) single day treatment.

Parameters	*Control*	*Prolactin*	*Experimental progesterone*	*Prolactin and progesterone*
Total lipids	72.0±20.0	**53.0±17.0	84.0±20.0	80.0±22.0
Total phospholipid	17.0± 1.5	18.2± 1.0	16.8± 1.2	15.0± 1.1
Total cholesterol	8.2± 0.8	8.3± 0.5	*18.1± 1.0	*18.2± 1.0
Total glycerides	46.8±10.8	*26.5±12.2	49.1±11.3	46.8± 3.8
Free cholesterol	2.2± 0.2	2.3± 0.3	544.4± 0.6	*4.8± 0.7
Monoglycerides	13.8± 2.6	**6.0± 0.9	13.9± 3.2	10.0± 1.3
Diglycerides	12.9± 1.8	***8.8± 1.1	11.2± 1.8	12.7± 1.8
Triglycerides	20.1± 4.9	***11.7± 1.3	24.0± 3.8	24.1± 2.3

Values are mean ±S.D. of 10 animals/group.

* $P<0.001$

** $P<0.005$

*** $P<0.05$

TABLE IV : Changes in the fractions of phospholipids in cauda (expressed as *mg/g* tissue) single day treatment.

Parameters	*Control*	*Prolactin*	*Experimental progesterone*	*Prolactin and progesterone*
Phosphatidyl inositol and phosphatidyl serine	1.34±0.5	1.3±0.3	1.12±0.4	1.1±0.4
Lysophosphatidyl choline	1.31±0.4	**2.3±0.4	0.78±0.4	0.98±0.2
Lysophosphatidyl ethanolamine and spingomyelin	1.78±0.6	**2.87±0.8	1.57±0.5	1.32±0.3
Phosphatidyl choline	3 35±0.9	2.31±0.5	3.19±0.9	3.07±0.9
Phosphatidyl ethanolamine	1.34±0.5	2.00±0.4	2.62±1.0	1.66±0.4
Phosphatidyl glyceryl phosphate and phosphatidic acid	7.88±1.2	7.42±1.8	7.52±1.8	6.87±1.3

Values are mean ±S.D. of 10 animals/group.

** $P<0.05$

Contrary to the findings in caput, prolactin alone brought about an increase in lysophasphatidyl choline and ethanolamine ($P<0.05$).

DISCUSSION

The importance of epididymal lipids in sperm maturation is ill defined and controversial. A number of substances are secreted in the epididymal lumen and lipids were shown in considerable amount by histologic studies (5), that they are under hormonal control is well established (10). During the transit through the epididymis, the epididymal sperms have been found to lose esterified cholesterol and phospholipids (20). Though it is suggested that they are utilised by the sperms as energy sources (19), yet it is not known whether they are also resorbed by the epididymal epithelium. Many substances that are secreted by the epididymis are found to affect sperm maturation by altering the *milieu interna* of the epididymis (3). But the importance of epididymal lipids in sperm maturation as well as the effect of hormone on them is not clearly shown.

In the caput epididymis, progesterone caused an accumulation of lipids. This may be either because of an increased synthesis or as a consequence to degenerative changes of epithelial cells as has been observed in the uterus (2). That it is not a stimulatory response is quite clear since anti-androgenic effect of progesterone and its deleterious effect on sperm maturation has been well established (15). That it is again a degenerative response is clear from the fact that phospholipids were also increased. The increase in all fractions of phospholipids concomittant to a decrease in the precursor, phosphatidic acid clearly spells that progesterone affected an increase in these fractions through accumulation and not by synthesis. Prolactin when given alone did not evoke any marked response, but it is found to potentiate the deleterious effects of progesterone by causing an accumulation of cholesterol, glycerides and phospholipids in caput, as observed in the combined hormone treatment.

Prolactin stimulated the phospholipid synthesis at the expense of glycerides, which is a true androgenic response (13). Prolactin has been reported to facilitate testosterone effects within androgen dependent tissues (21). The present study reveals that both caput and cauda shows differential responses which may be due to the structural, functional and metabolic differences in these two segments (8).

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