J. Biosci., Vol. 7, Number 2, March 1985, pp. 191–195. © Printed in India.

Physiology of the epididymis and spermatozoa

M. RAJALAKSHMI

Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi 110 029, India

MS received 21 December 1984

Abstract. The epididymis is an ideal extragonadal target site to inhibit fertility in the male. Synthesis and secretion of constituents like sialic acids, protein and glycerylphosphoryl choline by the epididymal epithelium under androgen control provide an ideal fluid environment for sperm maturation. An optimal level of sialic acid secretion by the epididymal epithelium is needed to maintain functional integrity of sperm. The existence of specific androgen receptors in the epididymis and spermatozoa are related to their ability to metabolise androgens.

Keywords. Epididymis; sialoproteins; androgen requirement; steroid metabolism.

Introduction

Post-testicular maturation of spermatozoa taking place in the epididymis is now well recorded. The immotile and immature spermatozoa released from the testis acquire progressive motility and fertilising ability during their transit through the long tortuous epididymal duct. Considerable research efforts ongoing in various laboratories are aimed at elucidating the events accompanying sperm maturation and the role of the epididymis in creating an ideal fluid environment for this process to take place.

Efforts to evolve new compounds as male contraceptive agents centered mainly around steroidal or non steroidal agents. The possibilities of inducing genetic damage by such compounds have been a cause for concern. An ideal agent should have selective action on epididymal spermatozoa, either during their epididymal transit or during their storage in the cauda epididymidis rendering them immotile and/or unable to fertilise ova. Such a compound would have the advantage of being effective, safe, reversible and relatively more rapid in its anti fertility action. For the successful development of such a contraceptive, an understanding of the basic physiology of the epididymis, its contained spermatozoa and the process of sperm maturation is imperative.

Epididymal structure and function

The epididymial epithelium is composed of two main cell types *viz.,* the principal cell and the clear cell. The relative distribution of these cells along the epididymal duct differs between species. The long columnar principal cells with stereocilia were

Abbreviations used: GPC, glycerylphosphoryl choline; DHT, dihydrotestosterone; M_r, molecular weight.

implicated in the absorption of a major portion of rete testis fluid entering the initial segment of the epididymis and in the secretion of a variety of substances like glycerylphosphoryl choline (GPC), proteins, sialic acid and other organic constituents. The clear cells in the rat epididymis are subdivided into two types and are concerned with the secretion of either glycoproteins or glycolipoproteins (Anand Kumar *et al.,* 1980). The blood epididymal barrier, constituted by the zona occludens of the junctional complexes at the apical ends of the principal cells (Hoffer and Hinton, 1984) also appears to play an important role in maintaining a physiological milieu in the epididymal canal suitable for sperm maturation.

The epididymal plasma is hyperosmotic (Howards *et al.,* 1979). Secretion of various organic constitutents by the epididymal epithelium like sialic acids, proteins and GPC or absorption from blood stream (*eg.* carnitine) may account for this. Sialic acids, either free or bound to proteins (Rajalakshmi and Prasad, 1968; Rajalakshmi *et al.,* 1973; Karkun *et al.,* 1974; Rajalakshmi *et al.,* 1975, 1976), are secreted by the epididymis in a number of species. The caput and cauda epididymides of the albino rat show a gradual increase in the synthesis of sialic acids during the pubertal period. A peak in synthetic activity occurs between days 40–45 of age before the entry of the first wave of spermatozoa into these regions (Rajalakshmi and Prasad, 1969). Coinciding with the entry of spermatozoa into the epididymis, sialic acid levels decrease perhaps indicating the role of sialoproteins in sperm maturation. In a comparative analysis of the changes in sialoproteins during sperm maturation in rodents and monkeys, Prasad and Rajalakshmi (1976), reported a decrease in sialic acids bound to spermatozoa during their transit from caput to cauda epididymidis. This was later corroborated by Toowicharanont and Chulavatnatol (1983) who reported decrease in neuraminidase sensitive sialic acids during sperm maturation. One of the complex changes taking place during epididymal transit of the spermatozoa was the increase in net negative charge on sperm surface (Bedford and Cooper, 1978) and bound sialic acid was responsible for the enhanced net negative charge on sperm surface (Nicolson *et al.,* 1977). However, in studies using whole spermatozoa (Prasad and Rajalakshmi, 1976; Toowicharanont and Chulavatnatol, 1971), it was demonstrated that acrosomal sialic acids contribute a major portion of total sialic acids. Epididymal maturation being accompanied by reduction in acrosomal size (Bedford, 1965) as well as by loss of acrosomal components (Holt, 1980), it was logical to suggest that total sialic acids of the spermatozoa would decrease. Coating of sialic acids to sperm surface during their epididynal transit resulting in an increase in net negative charge accounted only for a fraction of total sialic acids in the sperm.

Androgen dependency of epididymal function

The secretions of sialic acids by the epididymis is an androgen dependent event. Castration markedly reduced sialic acid levels of the epididymis to undetectable levels (Rajalakshmi and Prasad, 1968) but the normal levels could be maintained by exogenous androgen administration to castrated animals (Gupta *et al.,* 1974; Karkun *et al.,* 1974; Dinakar *et al.,* 1974 a, b). An optimal level of sialic acid secretion by the epididymal epithelium appeared to be a prerequisite for the maintenance of the

functional integrity of the sperm since inhibition of epididymal secretion by treatment with lutinizing hormone antiserum or by the androgen antagonist cyproterone acetate induced corresponding reduction in sialoproteins in epidydmal plasma and the spermatozoa (Rajalakshmi and Prasad, 1977; Gupta, *et al.,* 1974; Bose *et al.,* 1975). These results clearly indicate the existence of a functional inter-relationship between sperm maturation and the changes in sperm sialic acid.

While it was established that the functional integrity of the epididymis was dependent on androgens (Rajalakshmi and Prasad, 1968), subsequent studies also showed that the epididymis in rodents and monkeys had a higher androgen requirement than the accessory glands (Rajalakshmi and Prasad, 1968; Gupta *et al.,* 1974; Karkun *et al.,* 1974; Dinakar *et al.,* 1974a,b). Low doses of testosterone or dihydrotestosterone (DHT) maintained the secretory functions of accessory glands in rat, hamster and monkey while higher doses are required to maintain the secretory activity of the epididymis. These pioneering studies thus established the existence of a differential androgen requirement by the epididymis in comparison to the accessory glands. The greater androgen requirement of the epididymis may be related to the dual nature of androgen supply to this organ. Epididymis receives androgens from both the peripheral circulation and from the rete testis fluid from where it is transported to the epididymis in association with androgen binding protein. These androgens are bound to receptors in the epididymal cells, translocated to the nucleus manifest their effects. Presence of high affinity sites for androgens was demonstrated in rhesus epididymis (Rajalakshmi *et al.,* 1976). These receptors are inhibited by antiandrogens like cyproterone acetate. If antiandrogens with selective action on epididymal androgen receptors are available, these could be used to selectively alter epididymal milieu and the spermatozoa in the epididymis and thus induce specific antifertility action.

Epididymis secretes into its luminal environment specific proteins/glycoproteins which coat the surface of spermatozoa during their epididymal transit promoting maturation of spermatozoa and induction of forward motility. These proteins are synthesised by the epididymis during the pubertal period (Singh *et al.,* 1977) but the adult epididymal protein profile is evident only after the entry of the first wave of spermatozoa into the epididymis (Rajalakshmi, R., unpublished results). The synthesis and secretion of these epididymal proteins are under androgen regulation (Blaquier and Calandra, 1973; Rajalakshmi, 1984). The perfusate of the cauda epididymidis contains a glycoprotein of 32,000 molecular weight (*Mr*) (Wong *et al.,* 1981) which is concentrated in the cauda epididymidis and were present only on mature caudal spermatozoa and not on immature caput sperm. These results indicated an interaction between spermatozoa and the luminal plasma during their epididymal transit. A 38,000 *Mr* protein present on ejaculated human spermatozoa from men of proven fertility was absent on immotile sperm obtained from infertile human semen. But such infertile spermatozoa acquired this protein on incubation with normal human sperm and became motile (Wong *et al.,* 1982). These results indicated that epididymal proteins played an important role in human sperm maturation.

The epididymis, in addition to possessing androgen receptors mediating androgen action, was shown to be capable of metabolising steroids like testosterone. The major product of testosterone metabolism in most species is dihydrotestosterone (Gloyna and Wilson, 1969). Androgen action on epididymis was mediated through the conversion of

testosterone to DHT. Testosterone metabolising capacity of the epididymis was decreased by chronic exposure to antiandrogens like cyproterone acetate (Rajalakshmi and Prasad, 1976). Epididymal and ejaculated spermatozoa of a number of species also possessed enzymes capable of metabolising steroids (Hammerstedt and Amann, 1976). The preferred androgen substrate for spermatozoa and the major metabolites formed were species dependent. However, in a majority of rodents and lagomorphs, testosterone was preferentially converted to androstenedione (Rajalakshmi *et al.,* 1978; Seamark and White, 1964). Spermatozoa from primate species like rhesus monkey and human also convert testosterone to adrostenedione but the reverse reductive pathway was not functional (Rajalakshmi *et al.,* 1983). Estradiol 17-*ß* found in large quantities in seminal fluid was also metabolised to estrone by monkey and human spermatozoa (Rajalakshmi *et al.,* 1983). While the role of these metabolites in sperm physiology is not known, receptors for steroids were localised on sperm surface (Cheng, *et al.,* 1981 a, b). Infertile human spermatozoa appear to have a different steroid metabolising enzymatic machinery than normal fertile spermatozoa and convert testosterone to DHT and to androstenedione (Rajalakshmi, R., unpublished observations) unlike the normal human sperm. The reason for this is not known.

The epididymis as a target for contraceptive action

The epididymis, thus being intimately involved in spermatozoa acquiring motility and fertilising ability offers an ideal site for action of antifertility agents. Compounds with selective action on epididymal spermatozoa have the advantage of rapidity of action with no disadvantages of genetic damage to germ cell elements caused by anti-spermatogenic agents.

α-Chlorohydrin and the chlorosugars were extensively investigated for their specific antifertility action on epididymal spermatozoa and showed initial promise. However, their subsequent neurotoxic effects precluded their use in human clinical trials. Gossypol, with primary action on testicular germ cells, is also known to act on epididymal spermatozoa. This indicated that the site of action of gossypol is dependent on the dosage and duration of treatment. Since it is now known that the antifertility effect of gossypol is located in the levorotatory isomer, it is likely that lower doses of (–) gossypol would be sufficient to cause antifertility effects. Whether such low doses can induce selective epididymal effect is still to be worked out.

References

Anand Kumar, T. C. Prakash, A. and Prasad, M. R. N. (1980) *Anat. Anz.,* **147,** 220.

Bedford, J. M. (1965) *J. Anat.,* **99,** 891.

Bedford, J. M. and Cooper, G. W. (1978) in *Membrane Fusion* (ed G. Poste, and G. L. Nicolson), (Elsevier/North Holland Biomedical Press, Amsterdam), 65.

Blaquier, J. A. and Calandra, R. S. (1973) *Endocrinology,* **93,** 51.

Bose, T. K., Prasad, M. R. N. and Moudgal, N. R. (1975) *Indian J. Exp. Biol.,* **13,** 8.

Cheng, C. Y., Boettcher, B., Rose, R. J., Kay, D. J. and Tinneberg, H. R. (198 1a) *Int. J. Androl.,* **4,** 1.

Cheng, C. Y., Rose, R. J. and Boettcher, B. (1981b) *Int. J. Androl.,* **4,** 304.

Dinakar, N., Arora, R. and Prasad, M. R. N. (1974a) *J. Endocrinol.,* **60,** 399.

Dinakar, N., Arora, R. and Prasad, M. R. N. (1974b) *Int. J. Fertil.,* **19,** 133.
Gloyna, R. E. and Wilson, J. D. (1969) *J. Clin. Endocrinol.,* **29,** 970.
Gupta, G., Rajalakshmi, M. and Prasad, M. R. N. (1974) *Steroids,* **24,** 575.
Gupta, G., Rajalakshmi, M., Prasad, M. R. N. and Moudgal, N. R. (1974) *Contraception,* **10,** 491.
Hammerstedt, R. H. and Amann, R. P. (1976) *Biol. Reprod.,* **15,** 686.
Hoffer, A. P. and Hinton, B. T. (1984) *Biol. Reprod.,* **30,** 991.
Holt, W. V. (1980) *Biol. Reprod.,* **23,** 847.
Howards, S., Lechene, C. and Vigersky, R. (1979) in *The Spermatozoon,* (eds D. W. Fawcett and J. M. Bedford) (Baltimore-Munich: Urban and Schwarzenberg), p. 35.
Karkun, T., Rajalakshmi, M. and Prasad, M. R. N. (1974) *Contraception,* **9,** 471.
Koehler, J. K. (1981) *Arch. Androl.,* **6,** 197.
Nicolson, G. L., Usui, N., Yanagimachi, R., Yanagimachi, H. and Smith, J. R. (1977) *J. Cell Biol.,* **74,** 950.
Prasad, M. R. N. and Rajalakshmi, M. (1976) *Gen. Comp. Endocrinol.,* **28,** 530.
Rajalakshmi, M. (1984) *Arch. Andol.,* (in press)
Rajalakshmi, M., Arora, R., Bose, T. K., Dinakar, N., Gupta, G., Thampan, T. N. R. V., Prasad, M. R. N., Anand Kumar, T. C. and Moudgal, N. R. (1976) *J. Reprod. Fert. Suppl.,* **24,** 71.
Rajalakshmi, M., Reddy, P. R. K. and Prasad, M. R. N. (1973) *J. Endocrinol.,* **58,** 349.
Rajalakshmi, M., Reddy, P. R. K., Prasad, M, R. N., Upadhyaya, P. and Minocha, V. R. (1975) *Contraception,* **12,** 175.
Rajalakshmi, M. and Prasad, M. R. N. (1968) *J. Endocrinol.,* **41,** 471.
Rajalakshmi, M. and Prasad, M. R. N. (1969) *J. Endocrinol.,* **44,** 379.
Rajalakshmi, M. and Prasad, M. R. N. (1976) *Metabolism of testosterone by the accessory organs of the rat and its inhibition by cyproterone acetate,* presented at V Internat. Congr. Endocrinology, Hamburg.
Rajalakshmi, M. and Prasad, M. R. N. (1977) in *Reproductive Physiology,* (ed. R. O. Greep) (Baltimore University Park Press) p. 153.
Rajalakshmi, M., Leask, J. T. S. and Waites, G. M. H. (1978) *Steroids,* **31,** 747.
Rajalakshmi, M., Sehgal, A., Pruthi, J. S. and Anand Kumar, T. C. (1983) *Steroids,* **41,** 587.
Seamark, R. F. and White, I. G. (1964) *J. Endocrinol.,* **30,** 307.
Singh, R., Rajalakshmi, M. and Prasad, M. R. N. (1977) *Indian J. Exp. Biol.,* **15,** 825.
Towicharanont, P. and Chulavatnatol, M. (1983) *J. Reprod. Fert.,* **67,** 275.
Wong, P. Y. D., Tsang, A. Y. F. and Lee, W. M. (1981) *Int. J. Androl.,* **4,** 331.
Wong, P. Y. D., Tsang, A. Y. G. and Wang, C. (1982) *J. Androl.,* **3,** 6.