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Detection of aromatase, androgen, and estrogen receptors in bank vole spermatozoa

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Abstract

Spermatozoa are highly specialized cells which transport a single-copy haploid genome to the site of fertilization. Before this, spermatozoa undergo a series of biochemical and functional modifications. In recent years, the crucial role of androgens and estrogens in proper germ cell differentiation during spermatogenesis has been demonstrated. However, their implication in the biology of mature male gametes is still to be defined. Our study provides evidence for the first time that aromatase, the androgen receptor (AR), as well as the estrogen receptors α and β (ERα and ERβ), are present in bank vole spermatozoa. We demonstrated the region-specific localization of these proteins in bank vole spermatozoa using confocal microscopy. Immunoreactive aromatase was observed in the proximal head region and in both the proximal and distal tail regions, whereas steroid hormone receptors were found only in the proximal region of the sperm head. Protein expression in sperm lysates was detected by Western blot analysis. Immunohistochemical results were analyzed quantitatively. Our results show that bank vole spermatozoa are both a source of estrogens and a target for steroid hormone action. Moreover, the presence of aromatase and steroid hormone receptors in the bank vole spermatozoa indicates a potential function of these proteins during capacitation and/or the acrosome reaction.

Keywords: Spermatozoa; Bank vole; Aromatase; Androgen receptor; Estrogen receptors

1. Introduction

Spermatogenesis is a very complex and highly regulated process in which functional spermatozoa (haploid, 1n) are generated from primitive mitotic spermatogonia (diploid, 2n). During spermiogenesis, a series of morphologic remodeling occurs before the spermatozoa are released into the lumen of the seminiferous tubules [1]. In the sperm nucleus, DNA is either condensed to a single-copy genome that makes normal functions, such as transcription and translation, either impossible or massively reduced. Changes in the sperm membrane and cytoplasm take place during the epididymal transit by the addition and deletion of several proteins and molecules [2,3]. Moreover, sperm are exposed to various components of the seminal plasma that adhere to the cell membrane during ejaculation and transit through the female genital tract leading to capacitation [4].

It is well known that the initiation, maintenance, and reinitiating of spermatogenesis depends on androgen action [5,6]. Testosterone and its metabolite dihydrotestosterone mediate a wide range of physiological responses by signaling through nuclear and membrane androgen receptors (ARs) [7]. In addition, the AR itself plays an important role in the feedback regulation of testosterone concentration. In the testis of mammals AR has been found to be expressed in somatic cells,

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whereas its localization in germ cells at different spermatogenic stages remains unclear and controversial. A series of studies indicates the presence of AR in human and rodent germ cells [8–12], although other reports show a lack of AR expression in these cells [13–19].

Besides androgens, estrogens are also implicated in the regulation of the development and maintenance of the male reproductive system [20,21]. Estrogen action is mediated by two distinct nuclear receptors: estrogen receptor (ER)α and ERβ, which exhibit species-specific localization patterns and the levels of expression. In addition, nongenomic effects mediated through membrane ERs have been demonstrated in several cell types, although such receptors are still poorly characterized [22]. Multiple studies have clearly shown that the main modulator of androgen/estrogen balance, aromatase, as well as ERα and ERβ, are expressed in specific cells of the testis, epididymis and vas deferens [23–25].

Recent studies revealed the presence of various proteins in spermatozoa, although their function is still not fully understood. To date, there has been more focus on the involvement of androgen and estrogen in the physiology of mature sperm. Aromatase, AR, ERα, and ERβ have been recently detected in human and boar sperm, raising the hypothesis that steroid hormones could also regulate sperm functional properties [26–31]. Interestingly, in rodents only the presence of ERβ has been demonstrated in elongating spermatids and spermatozoa [32,33]. Therefore, it is still questionable what the roles of aromatase and the steroid hormone receptors are in androgen/estrogen signaling in rodent spermatozoa. In light of these data, it seems interesting to investigate whether AR, ERα, and ERβ, as well as aromatase, are present in spermatozoa of a seasonally breeding rodent, the bank vole.

2. Materials and methods

2.1. Animals

Bank voles (*Clethrionomys glareolus*, Schreber) were obtained from our own colony (Department of Endocrinology, Institute of Zoology, Jagiellonian University in Krakow, Poland), which have been reared under 18 h light/6 h dark cycles for 10 generations. Bank voles, kept under long light conditions in the laboratory, show similar reproductive characteristics as wild animals during the breeding season because reproduction and hormonal activity are regulated by the length of the light cycle [34–36]. The animal rooms were maintained at a temperature of 18 °C and a relative humidity of 55 ± 5%. Voles were housed in polyethylene cages (42 cm × 27 cm × 18 cm) furnished with sawdust and wood shavings for bedding. A standard pelleted diet (LSM diet; Agropol, Motycz, Poland) supplemented with seeds of wheat or oat, red beet, apples, and water was provided ad libitum. The animals were sacrificed at the age of 60 days. Testes and vasa deferentia were excised. From vasa deferentia spermatozoa were isolated and sperm smears prepared. For analysis of protein expression sperm samples and testes (positive control) were frozen at −80 °C. Thirty animals (N = 30) were included in these analyses with three slides assessed per vas deferens.

2.2. Ethics of experimentation

Experiments were performed in accordance with Polish legal requirements, under the license given by the Local Ethics Committee at the Jagiellonian University in Krakow, Poland.

2.3. Isolation of spermatozoa

Sperm cell isolation was assessed as described by Styrna et al. [37] with some modifications. After gentle pressing of the vas deferens with forceps, the content was expressed into 0.2 mL of warm (37 °C) phosphate-buffered saline (PBS) at pH 7.2 and allowed to disperse. Small drops of suspensions of sperm cells were transferred to microscope slides and smears were carefully made. Randomly selected smears were stained with eosin solution (1% eosin Y in water) and allowed to dry. The sperm morphology was evaluated under a light microscope (Microphot, Nikon, Tokyo, Japan). The percentage of spermatozoa with normal morphology among 200 counted spermatozoa from each male (N = 30) was reported.

2.4. Immunofluorescent assay

Immunofluorescent labeling was performed on freshly made and air-dried sperm smears, prepared as describe above. Smears were carefully rinsed with Tris-buffered saline (TBS; 0.05 M Tris-HCl plus 0.15 M NaCl, pH 7.6) and fixed using absolute methanol for 7 min followed by acetone for 4 min both at −20 °C and allowed to air-dry. Next, sperm smears were rinsed in TBS containing 0.1% Triton X-100. Nonspecific binding sites were blocked with 5% normal goat serum for 30 min. Thereafter, smears were incubated overnight at 4 °C in a humidified chamber in the presence of primary antibodies: (1) a rabbit polyclonal antibody against human AR (1:2000; Santa Cruz Biotechnology, Santa Cruz, CA, USA); (2) a polyclonal antibody