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Seasonal variations of aromatase and estrogen receptors expression in the testis of free-ranging sand rats

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ABSTRACT

An increasing number of studies revealed the importance of estrogen in male reproduction. However, most research was conducted in laboratory rodents subjected to standardized environmental conditions. Therefore, seasonal regulations of estrogen pathways remain poorly understood under natural conditions. Using immunohistochemistry, the expression of several molecules involved in the functioning of testis (i.e. 17-β estradiol [E2], P450 aromatase, estrogen receptors ESR1, ESR2, and GPER1 [also known as GPR30]) were investigated in free-ranging fat sand rats, *Psammomys obesus*, during the breeding and resting seasons. Leydig cells showed a strong immunoreactivity for aromatase in the testis sampled during the breeding season only; however, E2, ESR1, ESR2 and GPER1 were present during both seasons. Sertoli cells showed a positive signal for E2 and ESR2 during the breeding season; though, all molecules, except GPER1, were present during the resting season. Spermatogonia were reactive for E2, ESR2 and GPER1 during the breeding season and for ESR1 and GPER1 during the resting season. During both seasons, spermatocytes-I presented a moderate reactivity for E2, ESR1, ESR2 and a strong reactivity for GPER1; aromatase was detected during the resting season only. Spermatids and spermatozoa were present exclusively during breeding season and were reactive for all molecules; except round spermatids that were negative for aromatase. The functioning of the testis depends on finely tuned stimulation and inhibition systems. Our results suggest that differential expression of aromatase, ESR1, ESR2, and GPER1 across cells types is involved in the seasonal activation/inactivation cycle of spermatogenesis in a free-ranging species.

1. Introduction

The testis is a multifaceted organ that exerts exocrine and endocrine reproductive functions, notably spermatogenesis and steroidogenesis. Both functions are interacting intimately to maintain fertility. The production of sperm is under hormonal regulations of the hypothalamo–pituitary–gonadal axis: testicular activity is under the control of LH and FSH that are in turn under the control of GnRH, with different feedback loops among the endocrine systems involved. This complex regulatory system enables organisms to adjust their reproductive effort (e.g. sperm production, development of secondary sexual characteristics, sexual behaviors) to environmental conditions and to their physiological status (Willmer et al., 2005). Thus subtle regulations of reproductive functions determine fertility but also influence individual fitness and population viability. Sexual steroids occupy a central role in these regulations. In male vertebrates, testosterone secreted by the Leydig cells (induced by LH-stimulation) is critical for spermatogenesis and it stimulates other reproductive traits (McLachlan et al., 1996). Consequently, this major androgen steroid attracted considerable attention, detailed information is available in many animal species (Goldey and van Anders, 2015). For example, besides the classical roles exerted on secondary sexual characteristics, the testis testosterone maintains the blood–testis barrier (Meng et al., 2005), induces meiosis and postmeiotic development of germ cells (Dohle et al., 2003;

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Holdcraft and Braun, 2004) and inhibits germ cell apoptosis (Singh et al., 1995).

However, different studies revealed the importance of estrogen hormones in males, notably in the functioning of the epididymis; but this hormone also influences the hypothalamo–pituitary–testis axis and the production of sperm through the modulation of the activity of the Sertoli, Leydig and germ cells (Hess et al., 1997; O'Donnell et al., 2001). Estrogens are notably involved in spermatogonial stem cell division (Miura et al., 1999), initiation and maintenance of spermatogenesis (Ebling et al., 2000), and they promote the survival of germ cells (Pentikäinen et al., 2000). Estrogen biosynthesis is catalyzed by a microsomal P450 aromatase complex responsible for the irreversible transformation of androgens into estrogens (Payne and Hales, 2004). In the reproductive tract of immature males, estrogens are essentially produced by Sertoli cells (Van der Molen et al., 1981), whereas in mature individuals they are found in germ cells, spermatozoa and Leydig cells (Payne et al., 1976; Levallet et al., 1998b; Carreau et al., 2006, 2007a,b). Two different nuclear estrogen receptors (ER) have been identified (ESR1, ESR2) with similar affinities for estradiol (Kuiper et al., 1997). The localization of estrogen receptors in testicular cells varies depending on the species, developmental stages of the cells and types of receptors (Abney, 1999; O'Donnell et al., 2001; Hess and Carnes, 2004). Recently, a new receptor that binds estradiol has been described: the G protein-coupled estrogen receptor (GPER) also named G protein-coupled receptor 30 (GPR30, also known as GPER1). This receptor mediates the 17β-estradiol activation of various downstream signaling pathways and it exerts multiple roles in the cell physiology. Using ligands such as ER antagonist fulvestrant (ICI 182,780) it has been shown that GPER can be found in a large range of human and rodent tissues, more precisely in the endoplasmic reticulum compartment (Sirianni et al., 2008; Prossnitz and Barton, 2009). Importantly, 17β-estradiol may have differential effects when activating ESR (nuclear) versus GPER (non-nuclear) receptors. For example, the selective activation of GPER has a rapid anti-apoptotic effect in cultured Sertoli cells, whereas delayed cell proliferation can occur under ESR1 activation in other cells (this response takes time from gene activation to protein production). Nevertheless, these two different regulatory pathways are partly overlapping and they may involve cross-talk between the two receptors (Lucas et al., 2010).

Overall, a better understanding of the reproductive endocrinology of mammals necessitates considering both androgen and estrogen regulations in females and in males (Pelletier et al., 2000; Zhou et al., 2002). Although most comparative studies focused on circulating levels of sex steroids in mammals (Bronson, 1989), examination of the localization of their respective receptors is equally essential to accurately identify their main sites of action. Assessment of the localization of the major enzymes involved in steroidogenesis is also important (Payne and Hales, 2004). Research on these issues concentrated in laboratory rodents (Couse and Korach, 1999). Yet, histological studies of male reproductive tracts showed considerable variations among closely related rodent species (mice vs rats) in the localization and in the expression of sex steroid receptors for example (Zhou et al., 2002). Therefore, inferring possible regulation during the reproductive cycle in free ranging species of rodents from the limited laboratory studies available might be speculative. Especially when the strong variability in life history traits exhibited across the high phylogenetic diversity of mammals is considered (Bromham et al., 1996). Information gathered in free-ranging species of mammals subjected to seasonal variations is needed to assess the general validity of the results obtained in strains of rodents that have escaped natural selection over many generations (Schön and Blottner, 2008; Oliveira et al., 2009; Beguelini et al., 2014; Zarzycka et al., 2016).

Captivity investigations performed in seasonal breeder rodents (voles, hamsters) showed that moderate exposure to estradiol prompted gonadal recrudescence and spermatogenesis in individuals maintained under unfavorable photoperiodic regime (Pak et al., 2002; Gancarczyk et al., 2004). In domestic stallions (pseudo-seasonal breeders) capacitation and acrosome reaction were independent from a marked seasonal expression of estrogen receptors (ESR1, ESR2 and GPER), suggesting that upstream processes such as epididymal maturation were more important (Gautier et al., 2016). These captivity studies indicate that seasonal variations represent an appropriate system to identify and thus assess the roles of estrogens in the functioning of the testis in free ranging animals, as shown in a tropical bat species (Beguelini et al., 2014).

In this study, we examined adult males of the fat sand rats (*Psammomys obesus*) during the reproductive cycle. This species lives in an extremely arid environment characterized by drastic seasonal changes of the climatic conditions; breeding period occurs from autumn through early spring; a non-breeding resting phase takes place from late spring through summer. This contrasted seasonal pattern offers an opportunity to compare reproductive traits between sexually active versus inactive individuals. The reproductive physiology of fat sand rat has been intensively studied throughout the whole annual cycle, providing baseline information to examine possible changes in the expression of molecules involved in the estrogenic dependent functioning of testis. Seasonal variations of the genital tract (e.g. including seminal vesicles), of the histology of the testis (including indicators of hormonal activity), and of plasma levels of testosterone have been described (Khammar and Brudieux, 1984, Khammar, 1987; Gernigon et al., 1991; Gernigon, 1992; Menad, 2008, 2015; Menad et al., 2014). During the annual cycle, the testis of male fat sand rat undergoes profound changes. In the active period, sperm production is abundant and Leydig cells have a highly developed endoplasmic reticulum (a key substratum for GPER1). In the resting period, there is a reduction in the diameter of the seminiferous tubules, a stoppage of the spermatogenesis, and a regression of the endoplasmic reticulum of the Leydig cells associated with an accumulation of the lipids in the Leydig cells. Regarding testicular contents of androgens (ng/g of testis), high values were observed in autumn and in winter (testosterone: 7.6 ± 1.1; androstenedione: 0.76 ± 0.11) whereas low values were reported in early summer (June) (testosterone: 1.5 ± 0.3; androstenedione: 0.20 ± 0.05) with raising values in late July (Khammar and Brudieux, 1984). Annual variations of the testosterone metabolic clearance rate (liters/24 h/100 g body wt) were parallel to the changes of testicular androgens concentrations; clearance peaking in winter (6.7 ± 0.7) and decreasing in June (3.2 ± 0.3). Presumably this contrasted pattern of androgen production during the testis cycle should be reflected by marked seasonal differences in the expression of endocrine receptors.

The main aim of this study was to provide information on estrogen regulation in the testis in males of a wild species subjected to natural seasonal fluctuations. Indeed, although potential important roles of estrogen are suspected in free-ranging males of mammals, current information is limited to individuals maintained in captivity (Hamster, bank vole, roe deer), and two free-ranging bat species (Schön and Blottner, 2008; Oliveira et al., 2009; Beguelini et al., 2014; Zarzycka et al., 2016). In this study, we focused on the testis and on the localization of estradiol (E2), estrogen receptors (ESR1, ESR2, and GPER1) and of the P450 aromatase. Considering the meager level of scientific knowledge on these issues in wild species, we addressed a simple question. Do the expression and the localization of the main estrogen receptors and P450 aromatase vary among the main cell types (Leydig, Sertoli, germ cells) and during the breeding cycle?

2. Material and methods

2.1. Animals and samples

The fat sand rat (*Psammomys obesus*) is a diurnal rodent that lives in the North-West of the Algerian Sahara. This species has been used to study the seasonal changes in the reproductive cycle of arid desert