Testicular Estrogens and Male Reproduction

Serge Carreau and Jérôme Levallet

Besides somatic cells, aromatase gene expression and its transduction in an active protein in germ cells provides evidence of an additional site for estrogen production within testes of some mammals. Together with the widespread distribution of estrogen receptors in testicular cells, these data illuminate the hormonal regulation of male reproductive function.

In the male gonad, gonadotropins and testosterone, together with numerous intratesticular modulators, are responsible for the induction and/or the maintenance of spermatogenesis. For a long time, estrogens have been considered a specific female hormone; however, the presence of estrogens in the male gonad is well documented. The cytochrome P-450 aromatase (P450arom) is a product of a unique gene called CYP 19; it belongs to the cytochrome P-450 gene superfamily, which contains >481 members belonging to 74 gene families (14). The human CYP 19 is the only member of the 19th family, located in region q21.1 on chromosome 15. This gene stretches to >75 kb in length and is composed of 18 exons, 9 of them being translated. In addition, the gene includes eight noncoding exons, all of them are in the 5′ region under alternative splicing control after their transcription downstream by an equivalent number of promoters, so-called tissue-specific promoters. As a matter of fact, these promoters are specific for different endocrine and/or paracrine regulating factors. Nevertheless, whatever the tissue, the human aromatase is a unique 55-kDa protein composed of 503 amino acids.

The aromatase is the terminal enzyme involved in the irreversible transformation of androgens into estrogens. This microsomal enzymatic complex is composed of a specific heme-glycoprotein (P450arom) that functions with a ubiquitous reductase as an electron donor. The P450arom plays a role in development, reproduction, sexual differentiation, and behavior, as well as in bone and lipid metabolism, brain functions, and diseases such as breast and testicular tumors. Indeed, it is difficult to find a tissue completely devoid of aromatase gene expression (for reviews see Refs. 2 and 14). In mammals, the P450arom is located in the endoplasmic reticulum in almost all tissues but mainly in brain, gonad, placenta, and adipose tissue of humans and primates. In the male gonad, aromatase has been immunohistolocalized in Leydig cells of rat, boar, ram, stallion, and human. Whatever the age, aromatase activity has been measured in immature and mature rat Leydig cells as well as in Sertoli cells, whereas in pig, ram, and human the aromatase is mainly present in

S. Carreau and J. Levallet are in the Biochemistry Laboratory, IRBA, University of Caen, France.
Effects of estrogens on testicular functions

Synthesis of steroids and sperm production represent the main characteristics of the mammalian testes. These functions are controlled by gonadotropins whose specific actions are fine tuned via local factors produced by the testicular cells, and among them estrogens seem to play a crucial role.

To exert their effects, testicular or locally produced estrogens interact with specific estrogen receptors (ER), which in turn modulate the transcription of specific genes involved in cell growth, function, and differentiation. For over 10 years the only available data about estrogen roles were related to the existence of ER-α, but in 1996 a novel estrogen receptor called ER-β was cloned from a human testis cDNA library (for review, see Ref. 2). Therefore, the distribution of the two types of mRNA (ER-α and ER-β), as well as the protein in the male rat gonad, has been extensively studied, and most of the rat testicular cells, particularly gonocytes, pachytabe spermatocytes, and spermatids, contained ER-β (2, 12).

These new observations led scientists to carefully reevaluate the effects of estrogens (or antiestrogens) on the male reproductive tissues. In that respect, it has been shown that in vitro the multiplication of rat gonocytes is in part regulated by estradiol, which fits well with the presence of ER-β in these cells (12). Estrogens also seem necessary for the achievement of fertility of the male rodent (8); in fact, there is evidence in mouse, from estrogen receptor gene knockout (ERKO) experiments, that estrogens are involved, because in the adult ERKO mice seminiferous tubes are collapsed and contained few germ cells. It is worth noting that the luteinizing hormone and follicle-stimulating hormone levels in blood of wild-type and ERKO males are not statistically different, suggesting a likely local defect in ERKO (for review see Ref. 3). Indeed, Hess et al. (6) have demonstrated that the lack of fluid reabsorption in the proximal compartments of the epididymis leads to an accumulation of fluid within seminiferous tubules and therefore induces an increase in pressure that will in turn destroy germ cells. Recently, Sharpe and colleagues have reported that estrogens, through the modulation of aquaporin-1 expression, are involved in the regulation of fluid reabsorption in the proximal regions of the rat and monkey epididymides. In addition, estrogens are involved not only in some regulating steps of spermatogenesis of mouse but also through, for instance, the cadherin synthesis that mediates Sertoli-cell interactions. In terms of the germ cell development, it is known that estradiol plays a role in the reinitiation of spermatogenesis in the bear after the winter rest, and in the ram, the estradiol concentration in the testicular vein is positively correlated with the daily production of leptotene primary spermatocytes/testis. Moreover, the spermatid number and maturation are decreased after injection of either aromatase inhibitors or antiestrogens in rodents and primates (for review see Ref. 2).

Finally, the existence of male mice deficient in aromatase (ArKO) has helped to clarify the physiological role of estrogens (5). Briefly, the animals develop normally and the genital tract is anatomically in the control range when compared with wild-type mice. The males are able to breed and produce litters; however, from the age of 5 mo onward, some of them
It starts to have failure of spermatogenesis, and by the age of 1 yr all male mice develop abnormal spermatogenesis. A blockage of germ cell maturation at the spermatid stage (round and elongated spermatid numbers are decreased by 50%) compared with wild-type mice is observed without any change in the blood follicle-stimulating hormone levels (11). According to these findings, it is quite difficult to argue about the absolute requirement of estrogens in the spermatogenic process. Even though the evidence is in favor of a role of these female hormones, estrogen-targeted genes are still missing, especially during the germ cell maturational changes.

Testicular estrogens and human reproduction

Concerning the aromatase in human testis, the Leydig cells have long been considered a main source of estrogens. Later, from in vitro studies, we have shown that both Leydig cells and Sertoli cells produce estrogens. In addition, the Sertoli cell aromatase activity is under germ cell control, which is also observed in Sertoli cells from testes of prepubertal boys with Peutz-Jegler syndrome. Moreover, malignant germ cells have the capacity to produce estrogens (for review see Ref. 2).

These reports are in keeping with the observations showing that the concentration of estrogens in the rete testis fluid of men is far higher than in the peripheral blood. The regulation of P450arom expression has been little studied in human testis; the promoter II is mainly expressed (for review see Refs. 2 and 14), and a new promoter I.6 has been described that is overexpressed in testicular tumors (13).

The ER-β has for the first time been cloned in human testis, and Gustafsson and colleagues have demonstrated by in situ hybridization that ER-β is mainly located in round spermatids and to a lesser degree in pachytene spermatocytes (4). It has recently been reported that human sperm membranes contain a functional estrogen receptor (10).

Overall, these data are likely related to the following reports. First, the aromatase deficiency in men consecutive to a P450arom gene mutation leads to sterility (1) with >1 million spermatozoa/ml. An inactivating mutation in the ER-α gene (exon 2) has been reported by Smith et al. (15). In this mutation, the number of spermatozoa was normal, whereas the viability was decreased and the patient was infertile. Second, a correlation between the amount of estradiol in the seminal plasma and the germ cell number has been demonstrated, as well as a positive role for estradiol in improving spermatozoa migration. In contrast, high amounts of estrogens are deleterious for spermatogenesis, and an involvement of xenoestrogens has been invoked to explain the decrease of sperm counts in men (for review see Refs. 2 and 12). Whether these observations are related to direct and/or indirect (i.e., pituitary) effects of estrogens remains uncertain.

Conclusion

Together with Leydig cells, adult rat germ cells are able to express P450arom mRNA, which is translated as a biologically active enzyme involved in estrogen production (Fig. 1). Consequently, germ cells not only produce estrogens but contain estrogen receptors as well, which would explain part of the role (autocrine and/or paracrine) of estrogens in male germ cell development. The mechanism of action of estrogens in the reproductive organs of the male remains to be clarified, as well as the regulation of aromatase gene expression, especially in germ cells during testicular development.
Nevertheless, we have begun to understand the physiological roles (as well as the pathological effects) of these female hormones in males, and, obviously, their involvement in several steps of sperm production and maturation. Thus it is anticipated that parts of male gonadal function are not only androgen regulated but also estrogen controlled in mammals.