The testicular aromatase: from gene to physiological role

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Received: 5 October 2001; accepted: 27 December 2001

SUMMARY

The aromatase is the terminal enzyme responsible for estrogen biosynthesis. It is present in the endoplasmic reticulum membrane of steroidogenic cells. The aromatase gene is unique and its expression is regulated in a tissue and more precisely, in a cell-specific manner via the alternative use of various promoters located in the first exon I. Physiological role of estrogens in the regulation of mammalian testicular functions is indicated by: 1/the aromatase gene expression and its transduction in a fully active protein in somatic cells as well in germ cells of testes, 2/ the widespread distribution of estrogen receptors (ERα and ERβ) in the genital tract of the male. Our recent data showing that human ejaculated spermatozoa expressed specific transcripts for P450arom support and expand the observations reported in germ cells of other mammalian species. Therefore, female hormones (or the ratio androgens/estrogens) do play a role, either directly on germ cells or via testicular somatic cells, in the maintenance of male gonadal functions. Several steps, including spermatid production, the sperm maturation and/or survival provide opportunity for estrogen action. Reproductive Biology 2002 2 (1): 5 – 12

Key words: aromatase, estrogens, male gonad, spermatogenesis, estrogen receptors, reproduction, fertility

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The aromatase: from gene to estrogen production

The aromatase plays a role in development, sexual differentiation, reproduction and behaviour, but also in bone and lipid metabolism, brain function and cancers. Indeed, it is difficult to find a tissue devoid of any aromatase gene expression.

In the mammalian testis, gonadotropins and testosterone together with locally-produced factors are responsible for the induction and/or the maintenance of spermatogenesis. Estrogens have been considered for several decades as a specific female hormone; however the presence of these female hormones in the testis is well documented [4]. The cytochrome P450 aromatase is involved in the irreversible transformation of androgens into estrogens; it is a microsomal enzymatic complex composed of a specific heme-glycoprotein (P450arom) which functions with a ubiquitous reductase as an electron donor. The human P450arom is the product of a unique gene called \textit{CYP19} which belongs to the cytochrome P450 gene family containing more than 500 members [27]. This gene measures more than 100 kb length and is composed of 17 exons, nine of them being translated (fig. 1). In addition, the gene includes eight non-coding exons I located in the 5' end and controlled by alternative splicing through tissue-specific promoters which are themselves submitted to regulations [28]. In humans, whatever the tissue, the aromatase is a unique protein of 55 kDa composed of 503 amino acids.

\textbf{Fig. 1.} The human cytochrome P450 aromatase gene. The size of the gene located on chromosome 15 is longer than 100 kb. In dark are shown the coding exons (I - X); the untranslated exons I are presented on the gene with their promoters except for exon I.5. The length of each exon is given; (/): unknown size; ATG: transcription starting site; HBR: heme binding region and polyadenylation sites are located on the gene.
In the male gonad the aromatase has been immunohistolocalized in Leydig cells. However, the aromatase activity has been demonstrated in both rat Leydig cells and Sertoli cells, whereas in pigs, rams and man this enzyme activity seems to be localized solely in Leydig cells [4]. More recently, in testes of rodents, birds and bears it has been reported that besides somatic cells, germ cells represent an additional source of estrogens [5]. In rats, the amount of P450arom transcripts is twice as high in pachytene spermatocytes as in round spermatids whose level is 20 times higher than in spermatozoa [18]. Moreover, using polyclonal antibodies against human placental aromatase we have shown that not only Leydig cells but also elongated spermatids are strongly positive [18] as also reported for spermatozoa within the epididymis [14]. Conversely, the aromatase activity, likely located in the cytoplasmic droplet of spermatozoa [14], is four to five times higher in testicular spermatozoa than in either pachytene spermatocytes or spermatids (all together the aromatase activity in rat germ cells accounts for over 60% of the total testicular aromatase activity; [18]). In addition, we have reported the existence of alternative splicing events of P450arom mRNA in rat pachytene spermatocytes and round spermatids which leads to putative P450arom isoforms unable to convert androgens into estrogens [19]. It is noteworthy that in rat Leydig cells, whatever the age, the level of P450arom mRNA is almost identical. By contrast in mature rat Sertoli cells the amount of these transcripts is 10 times lower than that in Sertoli cells of 20 days-old animals which is likely due to the negative effect exerted by germ cells on the Sertoli cell expression of the aromatase [4, 17].

Therefore in testes of some vertebrates, the aromatase gene is expressed and translated in a fully active enzyme not only in somatic cells but also in germ cells providing a new source of estrogens [5].

**Estrogens and spermatogenesis: is it a relevant feature?**

In order to exert a biological role estrogens should interact with estrogen receptors (ER) which in turn modulate the transcription of specific genes. Most of the available data about estrogen effects have been for more than 20 years related to the existence of only one type of estrogen receptor, ERα; however, in 1996 a second estrogen receptor termed ERβ was cloned [15]. Consequently, the distribution of the two types of ERs especially in the male gonad of mammals is currently re-examined. Most of the rat testicular cells, especially Sertoli cells, spermatogonia, pachytene spermatocytes and round spermatids contained ERβ. Estrogen receptor α was found to be located mainly in Leydig cells (table 1).
Tab. 1. Estrogen receptors (ERα and ERβ) and aromatase activity in the genital tract of adult rodents

<table>
<thead>
<tr>
<th>Tissue/Cell</th>
<th>ERα</th>
<th>ERβ</th>
<th>Aromatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leydig cell</td>
<td>+</td>
<td>+/—</td>
<td>+</td>
</tr>
<tr>
<td>Peritubular cell</td>
<td>—</td>
<td>+</td>
<td>+/—</td>
</tr>
<tr>
<td>Sertoli cell</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spermatogonia</td>
<td>—</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Pachytene spermatocytes</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Round spermatids</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spermatozoa</td>
<td>+?</td>
<td>+?</td>
<td>+</td>
</tr>
<tr>
<td>Rete testis</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Efferent ducts</td>
<td>+</td>
<td>+/—</td>
<td>ND</td>
</tr>
<tr>
<td>Epididymis</td>
<td>+/—</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>—</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Prostate</td>
<td>—</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not determined; +?: not characterized (α or β)

At least two steps of the spermatogenesis seem to be in part regulated by estrogens: the number of stem germ cells and the spermatid elongation. In vitro the rat gonocytes number is controlled by growth factors and estradiol [20]. There is evidence from estrogen receptor gene knock out (ERKOα) mice that estrogens are necessary for the full maturation of spermatozoa and, thus, for acquisition of fertilization ability [9]. In that context Hess et al [13] have demonstrated that the lack of fluid reabsorption in the proximal parts of the epididymis leads to an accumulation of fluid within seminiferous tubules, which in turn destroys the germ cells in the ERKOα mice. Indeed Fisher et al [11] have reported that estrogens via the modulation of aquaporin-1 expression, are likely involved in the regulation of fluid reabsorption in the proximal regions of rat and monkey epididymides.

Other data related to the germ cell development clearly favour the role of estrogens in the recrudescence of spermatogenesis such as in bears and squirrels (for review see: [4]). In the bank vole, a seasonal-breeder rodent, we have shown that photoperiod controls the expression of two androgen receptors, P450arom and ERs (α and β) in testicular cells [3]. More precisely, P450arom and ERβ are much more expressed in testes (namely spermatids) of long photoperiod-reared animals in which spermatogenesis is fully
developed when compared to short-day length bred animals with regressed testes [2]. In rams, estradiol concentration in the testicular vein is positively correlated with the daily production of leptotene primary spermatocytes/testis [8]. In addition, the number and the maturation of spermatids are diminished after the injection of either aromatase inhibitors or antiestrogens in rodents and primates [26]. Moreover, estradiol administration has been proven 1) to affect the development of spermatogenesis in mice deficient in gonadotropins ([10], together with a slight increase of blood FSH level); 2) to be responsible for shortening the time of the appearance of the first wave of spermatogenesis in rats [1].

Nevertheless, in ERβ knock out mice no abnormal development of germ cells has been observed and the males were fertile [9]. Finally, the data published by Mahato et al. [22] demonstrate that the germ cells from ERKOα mature correctly when they are transplanted in a wild type male testis which suggests that ERα is not absolutely required for germ cell development and that the sterility of the ERKOα males is likely related to some failure of testicular somatic cell functions.

Available today male mice deficient in aromatase (ArKO) have helped to clarify the physiological role of estrogens in the regulation of gonadal functions [24, and for review see: 23]. These animals develop normally and the genital tract is anatomically normal when compared to the wild-type. The males are able to breed and to produce litters, however starting at the age of 5 months onwards some of ArKO males present failures of spermatogenesis and by the age of one year all male mice develop abnormal spermatogenesis with a blockage of germ cell maturation at the spermatid stage (there is a 50% decrease of both round and elongated spermatid numbers) associated with an increase of apoptotic cells [25]. Conversely, transgenic male mice which overexpress aromatase are infertile with Leydig cell tumors [12]. These last data are in agreement with and extend the recently published results concerning another mice model overexpressing aromatase, in which cryptorchidism, Leydig cell hyperplasia, dysmorphic tubules and disrupted spermatogenesis have been described [21].

Conclusions

Now it is clear that not only testicular somatic cells (Leydig cells and Sertoli cells) but also germ cells are able to express P450arom mRNA which is translated in a biologically active enzyme involved in the transformation of androgens into estrogens [4-6]. Therefore, the androgen/estrogen ratio is modified in germ cells. If testosterone is involved in the regulation of testicular functions, estrogens are also necessary 1/ in the control of
gonadotropins secretion, 2/ in the modulation of the Leydig cell development and function, 3/ in development and/or maintenance of spermatogenesis and spermiogenesis in some mammals [7, 23].

In fact, germ cells (both meiotic and post-meiotic cells) do not only synthesize estrogens but contain estrogen receptors, too. This would explain, in part, the role (autocrine and/or paracrine) of estrogens in male germ cell development (fig. 2). The mechanism of estrogen action in the reproductive organs of the male, including the estrogen-targeted genes, remains to be studied. Nevertheless, we are beginning to understand the physiological role of female hormones as well as their pathological effects in males. Several steps, including production and epididymal maturation of spermatozoa provide opportunity for estrogen action. In that context it is important to emphasize our recent data\(^1\) [16] showing that in ejaculated human spermatozoa, the aromatase is also expressed and that the level of transcripts is correlated with the motility.

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\(1\) Lambard S Galeraud-Denis I Bourahima H Chocat A Carreau S Expression of cytochrome P450 aromatase in ejaculated human spermatozoa, in preparation
ACKNOWLEDGMENTS

I am greatly indebted to my collaborators: J. Levallet, C. Genissel, S. Bourguiba, S. Lambard, I. Denis and B. Bilinska.

REFERENCES


