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Review

Regulation and perturbation of testicular functions by vitamin A

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In addition to playing a fundamental role in very diverse processes such as vision and the growth and differentiation of numerous types of cell, vitamin A (retinol) and its principal biologically active derivative, retinoic acid, are clearly involved in the regulation of testicular functions in rodents. An excess of vitamin A leads to testicular lesions and spermatogenetic disorders, and a deficiency induces early cessation of spermatogenesis and adversely affects testosterone secretion. Furthermore, mice mutant for retinoic acid α receptors and retinoid X β receptors are sterile. Retinoids appear to exert an action on the three main testicular types of cell (Sertoli, germinal and Leydig cells), as they act on the signalling pathways and Sertoli cell metabolism, and modify numerous factors secreted in Sertoli cells. Retinoids also appear to be necessary for the proliferation and differentiation of A spermatogonia, and for spermiogenesis. In addition, vitamin A deficiency leads to atrophy of the accessory sex organs after decreased testosterone production. Recent studies have shown that retinoids already affect these three types of cell in fetuses. Curiously, the effects of retinoids on fetal and adult testis seem opposed.

Deficiency or excess of vitamin A or retinol is frequent in humans. Severe vitamin A deficiency can be observed in most developing countries (Mora *et al.*, 1998) and affects mainly children and pregnant women, whose needs for retinol are great (Gerster, 1997). In the industrialized countries, severe hypovitaminosis A is rarely found, but an insufficient nutritional intake of vitamin A has been reported in 20–25% of adult women in a study of the Paris region (Hercberg *et al.*, 1994). Conversely, hyper-retinoidaemia is frequently provoked for therapeutic purposes, as high doses of retinoids are used to treat skin diseases and several types of cancer, including cancers of the lung, kidney, skin and blood cells (Dragnev *et al.*, 2000). Oral retinoids such as tretinoin, isotretinoin or etretinate, designed to treat skin diseases such as severe acne and psoriasis, were available between the early 1980s and 1990s and the compliance of pregnancy avoidance policies after the end of treatments with these drugs has not always been respected. The first children born to women treated with these synthetic retinoids are reaching reproductive age but, as yet, no studies have been performed to investigate the effects of these treatments, which greatly increased the concentrations of retinoids sometimes for several months after the end of the treatment, on the fertility of this generation.

A normal diet should provide sufficient dietary vitamin A through the consumption of animal fat, eggs, butter and coloured fruit and vegetables containing beta-carotenes. Moreover, other products such as milk and cereals are artificially supplemented with vitamin A. The use of multivitamin supplements, particularly by women, is also common in the industrialized countries. These cumulative vitamin A intakes may increase retinol above recommended concentrations in subclasses of the population.

Although no study has yet been conducted to evaluate the importance of an alteration in vitamin A and retinoid intake in the development and maintenance of testicular functions in men, it has long been known that retinolaemia in rats and mice is involved in testicular functions but has no effect on ovarian functions. An excess of vitamin A causes testicular lesions and spermatogenetic disorders (Lamano Carvalho *et al.*, 1978). However, vitamin A deficiency induces early cessation of spermatogenesis (Wolbach and Howe, 1925), characterized by degeneration of all the meiotic germ cells (Thompson *et al.*, 1964; Morales and Griswold, 1987) and defective secretion of testosterone (Appling and Chytil, 1981), and can be compensated for by dietary vitamin A supplementation or injection of high doses of retinoic acid, the active metabolite of vitamin A (Thompson *et al.*, 1964; Appling and Chytil, 1981; Van Pelt and de Rooij, 1991).

Over the last 10 years, many studies have improved our knowledge of the location of retinoic acid receptors, the identification of their target genes and the involvement of retinoids in testicular development. The aim of this review is to present a synthesis of current knowledge on this question.

Retinoid acid receptors

Retinoic acid receptors belong to the superfamily of nuclear receptors of steroid and thyroid hormones, and include two

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Table 1. Localization of retinoic acid receptors (RARs) and retinoid X receptors (RXRs) in fetal, neonatal and adult testis

	Fetal testis	Neonatal testis	Adult testis
Sertoli cells	RARβ[3]; RARγ[3]; RXRγ[2]	RARα[4]; RARβ[4]; RARγ[2]; RXRα[2,4] ; RXRγ[2,4]	**RARα**[1,2,4]; RARβ[2,4]; RARγ[2,4]; RXRα[2,4]; **RXRβ**[2,4,5,6]; RXRγ[2,4]
Germ cells	Gonocytes: RARα[2,3]; RARβ[2,3]; RARγ[3]; RXRα[2]; RXRγ[2]	Gonocytes: RARα[2]; RARβ[2,4]; RARγ[2]; RXRα[2,4] ; RXRβ[2,5]; RXRγ[2,4]	Spermatogonia, spermatocytes and spermatids: **RARα**[1,2,4]; RARβ[2,4]; RXRα[2,4]; RXRγ[2,4,5] Spermatozoa: none[1,4,5]
Leydig cells	RARα[2,3]; RARβ[2,3]; RARγ[3]; RXRα[2]; RXRβ[2]; RXRγ[2]	RARα[2]; RARβ[2,4]; RARγ[2]; RXRα[2,4]; RXRβ[2,4]; RXRγ[2,4]	RARγ[2,4]; RXRα[2,4,,5]; **RXRβ**[2,4,5]; RXRγ[2,4,5]

Receptors the knockout of which induces sterility of testicular origin are shown in bold.
References: [1]Akmal *et al.*, 1997; [2]Boulogne *et al.*, 1999; [3]Cupp *et al.*, 1999; [4]Dufour *et al.*, 1999; [5]Gaemers *et al.*, 1998; [6]Kastner *et al.*, 1996.

main families: retinoic acid receptors (RARs) that bind all-*trans* and 9-cis retinoic acid isomers, and retinoid X receptors (RXRs) that preferentially bind the 9-cis isomer (for a review, see Giguère, 1994). Each family comprises three classes, α, β and γ, encoded by different genes. RAR can heterodimerize with the RXR or with other nuclear receptors to act specifically on the retinoic acid response elements and activate the transcription of target genes. RXR can homodimerize or heterodimerize with other transcription factors to bind specific DNA response elements.

The six classes of receptor have been located in rats and mice by immunohistochemistry or *in situ* hybridization in the different types of cell of the fetal, immature and adult testis (Huang *et al.*, 1994; Kastner *et al.*, 1996; Akmal *et al.*, 1997; Gaemers *et al.*, 1998a; Boulogne *et al.*, 1999; Cupp *et al.*, 1999; Dufour and Kim, 1999).

In the fetal or neonatal testis (Table 1), the gonocytes express the three RAR classes (-α, -β and -γ), as well as the RXR-α and -γ classes, but this expression changes throughout development and the location of the receptors is often cytoplasmic (Boulogne *et al.*, 1999; Cupp *et al.*, 1999). Immature Sertoli cells, which unlike adult Sertoli cells are mitotically active, express only RAR-β and -γ and RXR-α and -γ (Boulogne *et al.*, 1999); however, Dufour and Kim (1999) showed that immature Sertoli cells express RXR-α and -γ and also RAR-α and -β but no RAR-γ. This discrepancy may have occurred because of the use of different antibodies. Fetal Leydig cells, which form a generation of cells distinct from adult Leydig cells, express all three classes of RAR (Boulogne *et al.*, 1999; Cupp *et al.*, 1999) and all three classes of RXR (Boulogne *et al.*, 1999).

In the adult testis (Table 1), four classes of receptor have been identified in the germ cells: RAR-α and -β and RXR-α and -γ (Kastner *et al.*, 1996; Akmal *et al.*, 1997; Gaemers *et al.*, 1998a; Dufour and Kim, 1999). RAR-α is expressed essentially from the spermatocyte to the spermatid stage in the course of elongation, whereas RAR-β is expressed earlier, from the spermatogonia to the round spermatid stage. RXR-α and -γ are expressed at all these stages. The haploid germ cells no longer express any retinoic acid receptor from the elongated spermatid stage onwards. The adult Sertoli cells express all six classes of retinoic acid receptor, and the Leydig cells express all classes except RAR-α (Akmal *et al.*, 1997).

Thus, the distribution of retinoic acid receptors in the testis is very complex and often redundant, and depends not only on the type of cell but also on the stage of testicular differentiation and the spermatogenic stage. In addition, these receptors are sometimes located in the cytoplasm and are therefore inactive (Boulogne *et al.*, 1999; Dufour and Kim, 1999). The receptors may be transported into the nucleus in the presence of retinoic acid or according to other signals (Akmal *et al.*, 1997). Thus, for example, in the Sertoli cells, the nuclear location of RAR-α can be induced by retinoic acid and blocked by the action of FSH (Braun *et al.*, 2000). Similarly, the expression of retinoic acid nuclear receptors is not constant and may be subject to different types of regulation. In particular, in the testis of vitamin A-deficient animals, retinol increased the expression of RAR-α mRNA (Kim and Griswold, 1990; Akmal *et al.*, 1998) without changing the concentration of RAR-β mRNA (Kim and Griswold, 1990). Retinoic acid increases the expression of RAR-β (Gaemers *et al.*, 1997). These findings imply a complex model of signalling. However, only mice mutant for RAR-α or for RXR-β were rendered sterile by defective testicular functions (Lufkin *et al.*, 1993; Kastner *et al.*, 1996), indicating that these are probably the two most essential receptors.

Finally, the transcriptional activity of retinoid acid nuclear receptors may be modulated by interaction with other proteins acting as co-activators or co-repressors. These factors appear to act by modifying the acetylation of DNA histones, thus modifying the structure of chromatin and thereby preventing transcription. Many of the co-activators have histone acetyl transferase activity, whereas co-repressors either have histone deacetylase activity or are associated with other proteins that have histone deacetylase activity. In the absence of a ligand, the heterodimer RAR–RXR might be associated with a co-repressor such as the silencing mediator for retinoic acid and thyroid hormone receptors or the nuclear receptor co-repressor (Bernardini *et al.*, 1997; Leo *et al.*, 2001). Receptor activation

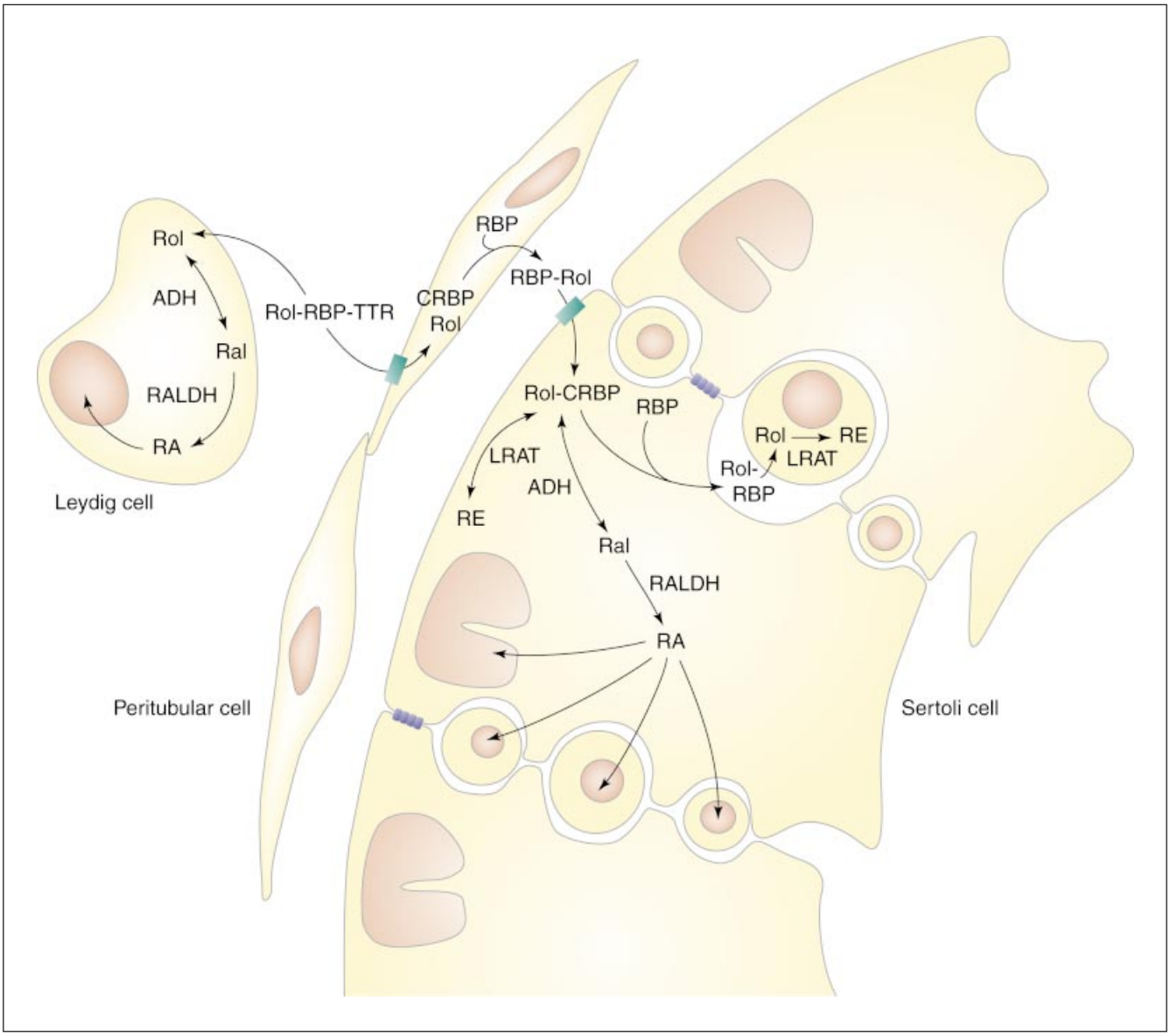

Fig. 1. Retinol metabolism in the adult testis. Circulating retinol (Rol) is bound to retinol binding protein (RBP), which is complexed with transthyretin (TTR). Retinol is internalized in the peritubular cells and then redistributed to the Sertoli cells. The Sertoli cells oxidize retinol into retinoic acid (RA) for their own needs and the needs of the germ cells, and also store retinol in the form of retinyl esters (RE). ADH: alcohol dehydrogenase; RALDH: retinal dehydrogenase; CRBP: cellular retinol binding protein; LRAT: lecithin–retinol acyltransferase; Ral: retinaldehyde.

by retinoic acid may dissociate the co-repressor complex and induce the recruitment of co-activators such as the steroid receptor co-activator 1, receptor-associated coactivator, cellular retinol-binding protein (CRBP) or thyroid hormone receptor-interacting protein (Bernardini *et al.*, 1997; Leo *et al.*, 2001). There may be a third category of coregulators acting as repressors on receptors bound to their ligand, as is the case for the receptor-interacting protein (Wei *et al.*, 2001). Unfortunately, in the testis, the expression of all these co-activators and co-repressors and their interaction with retinoid receptors is poorly documented.

Metabolism of retinol in the testis

It was thought that retinol exerts action in the testis but that retinoic acid exerts none (Thompson *et al.*, 1964). Injection of physiological doses of retinol, and not of retinoic acid, does restore normal spermatogenesis in vitamin A-deficient rats. However, Van Pelt and de Rooij (1991) showed that

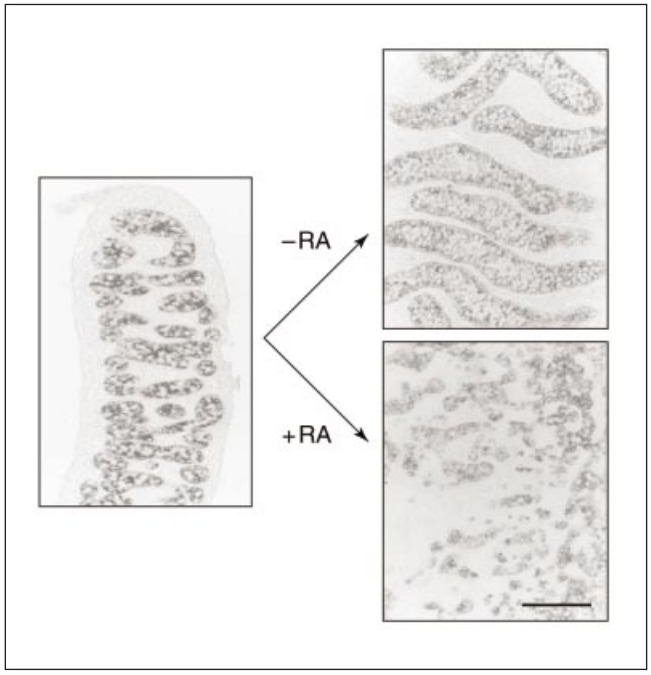

Fig. 2. Effect of retinoic acid (RA) on the organization of the seminiferous cords in testis of rat fetus. A fetal testis was explanted at day 14.5 after conception and cultured for 3 days in the absence or presence of 10^{-6} mol retinoic acid l^{-1}. At the time of explantation (14.5 days after conception) and after culture (14.5 + 3.0 days), Sertoli cells were identified by immunolocalization of anti-Müllerian hormone. Scale bar represents 100 μm.

spermatogenesis can be re-initiated by retinoic acid, provided that it is injected repeatedly at very high doses, indicating that the blood–testis barrier inhibits the passage of retinoic acid circulating towards the germ cells, and that the Sertoli cells synthesize retinoic acid from circulating retinol. This contention is supported by the fact that the passage of radioactive retinoic acid into the testis is inhibited compared with the passage of retinoic acid into other tissues (Kurlandsky *et al.*, 1995).

It is now known that the stages of the testicular retinoid metabolism are complex and involve different types of cell (Fig. 1). The first step in this metabolism takes place in the peritubular cells, which contain large quantities of CRBP, an intracellular protein that binds retinol with a high affinity (Blaner *et al.*, 1987). The peritubular cells take up the circulatory retinol bound to other transport proteins, such as retinol binding protein (RBP) and transthyretin (TTR), and secrete it as a complex formed with a new RBP, in the direction of the Sertoli cells (Davis and Ong, 1995).

CRBP is also present in Sertoli cells and its expression varies according to the stage of the cycle of the seminiferous epithelium, indicating that the need for retinol depends on the type of germ cells present (Blaner *et al.*, 1987; Schmitt and Ong, 1993). Sertoli cells are the main site of retinoic acid synthesis (Cavazzini *et al.*, 1996). Thus, the enzymes allowing retinol oxidation into retinoic acid (alcohol dehydrogenase and retinal dehydrogenase) are essentially located in the Sertoli cells (Deltour *et al.*, 1997; Zhai *et al.*, 2001). These cells may then 'distribute' the retinoic acid to their neighbours, notably to germ cells. Furthermore, production of retinol acid by Sertoli cells increases during testicular development. Sertoli cells are also the main site of retinol storage. They express lecithin–retinol acyltransferase (LRAT), which allows the esterification of retinol (Cavazzini *et al.*, 1996). FSH and retinoic acid increase retinol storage in the form of retinyl esters in Sertoli cells but retinol oxidation to retinoic acid is reduced by retinoic acid and increased by FSH (Guo *et al.*, 2001). However, the germ cells may themselves store retinol in the form of retinyl ester, because they also express LRAT, especially at the spermatid stage (Schmitt and Ong, 1993), and may also synthesize their own retinoic acid.

Leydig cells also express the enzymes necessary to convert retinol into retinoic acid (alcohol dehydrogenase (ADH) and retinal dehydrogenase) (Deltour *et al.*, 1997; Lopez-Fernandez and del Mazo, 1997; Hardy *et al.*, 2000; Zhai *et al.*, 2001). Several of the enzymes of retinoic acid metabolism may actually be using androgens as substrates in the testis (Hardy *et al.*, 2000).

Also present in the testis are cellular retinoic acid binding protein types I and II (CRABP), which bind retinoic acid to facilitate its transport to the nucleus or its catabolism in the different types of testicular cell, except for the peritubular cells (Blaner *et al.*, 1987; Faraonio *et al.*, 1993; Zheng *et al.*, 1996). However, these proteins do not appear to be essential because animals mutant for the two types of CRABP are normal in their development, fertility, lifespan and general behaviour (Lampron *et al.*, 1995).

Retinoids and testicular development

Retinoic acid causes disruption of the seminiferous cords in the testis of rat fetuses (Marinos *et al.*, 1995; Cupp *et al.*, 1999; Livera *et al.*, 2000) (Fig. 2) and also has numerous other effects on testicular development (Table 2). Cupp *et al.* (1999) showed that retinoids increased transcription of the three isoforms of transforming growth factor β (TGF-β) in cultured neonatal testicular cells.

Our group used an organotypic culture system to show that retinoic acid inhibits the stimulatory effect of FSH on the production of cAMP in rat Sertoli cells during fetal and neonatal life. The use of selective synthetic analogues of the different RAR and RXR revealed that this effect involves RAR-α (Livera *et al.*, 2001). Furthermore, after birth, retinoic acid increases the proliferation of Sertoli cells via RAR-β (Livera *et al.*, 2001) as well as their production of transferrin (G. Livera, unpublished).

Retinoic acid diminishes the proliferation of fetal and neonatal gonocytes by acting on both apoptosis and mitosis via the activation of RAR-α (Boulogne *et al.*, 1999; Livera *et al.*, 2000, 2001; B. Boulogne, unpublished). The knockout of RAR-α led to an increase in the number of germ cells in mouse fetuses and neonates, indicating the involve-

Table 2. Principal effects of retinoids on testicular cells

	Testis		
	Fetal	Neonatal	Adult
Sertoli cells	↓ Organization of cords[1–3] ↑ Transferrin[4] ↓ cAMP response to FSH[3]	↑ Mitosis[3] ↑ Transferrin[4] ↓ cAMP response to FSH[3] ↑ TGF-βs[1]	↑ c-jun, c-myb[6] ↑ Transferrin[7,8] ↓ cAMP response to FSH[9] ↓ PKC[10] ↑ Glycoproteins (Sgp-2)[11] ↑ ABP[8]; IGFBP-4[12] ↑ COX[13]; ornithine decarboxylase[14] ↑ Inhibin A[15] ↓ Plasminogen activator[16,17] ↓ Androgen receptor[15] ↑ PGD2-S[18] ↑ CRBP[19], RARα[20], RARβ[21]
Germ cells	↓ Gonocytes[3,5] ↑ Mitosis ↑ Apoptosis	↓ Gonocytes[5]	↑ Spermatogonia proliferation[22] ↑ Spermatid elongation[23]
Leydig cells	↓ Basal testosterone secretion[3]	Without relevant effect	↑ Basal testosterone secretion[24] ↑ StAR[25] ↑ P450C17[26] ↓ 3βHSD[26] ↓ LHR[26]

Abbreviations: TGF-β: transforming growth factor β; PKC: calcium-dependent protein kinase; ABP: androgen-binding protein; IGFBP: insulin-like growth factor-binding protein; COX: cytochrome *c* oxidase; PGD2-S: prostaglandin D2 synthetase; StAR: steroidogenic acute regulatory protein; P450C17: cytochrome P450 17α-hydroxylase–C17-20 lyase; 3βHSD: 3β-hydroxysteroid dehydrogenase; LHR: luteinizing hormone receptor.
References: [1]Cupp *et al.*, 1999; [2]Marinos *et al.*, 1995; [3]Livera *et al.*, 2000; [4]G. Livera, unpublished; [5]Boulogne *et al.*, 1999; [6]Page *et al.*, 1996; [7]Sigillo *et al.*, 1999; [8]Skinner *et al.*, 1989; [9]Galdieri and Nistico, 1994; [10]Galdieri *et al.*, 1986; [11]Guma and Bemard, 1994; [12]Bardi *et al.*, 1999; [13]Gaemers *et al.*, 1998; [14]Klamt *et al.*, 2000; [15]Zhuang *et al.*, 1997; [16]Rosselli and Skinner, 1992; [17]Canipari and Galdieri, 2000; [18]Samy *et al.*, 2000; [19]Eskild *et al.*, 1988; [20]Kim and Griswold, 1990; [21]Gaemers *et al.*, 1997; [22]Gaemers *et al.*, 1998; [23]Huang and Marshall, 1983; [24]Chaudhary *et al.*, 1989; [25]Lee *et al.*, 1999; [26]Lefevre *et al.*, 1994.

ment of the RAR-α receptor in the control of fetal gametogenesis, and implying that, in mice, circulating concentrations of retinoids exert a negative physiological effect on the onset of the germinal line (G. Livera, unpublished).

In rats, retinoids also reduced basal secretion of testosterone in fetal Leydig cells during differentiation of these cells (Livera *et al.*, 2000). However, in the presence of high doses of LH or hCG, retinoids stimulated testosterone secretion (G. Livera, unpublished). The moderate vitamin A deficit results in increased testicular steroidogenesis during fetal and neonatal life, showing that circulating concentrations of retinol exert a physiological inhibitory effect on the development of the endocrine function of the testis in rats (G. Livera, unpublished).

Retinoids and Sertoli cell functions

Retinoids are involved in the control of numerous functions in adult Sertoli cells (Table 2), the best documented of which is Sertoli cell secretion. Retinoids increase the secretion of transferrin, androgen-binding protein (ABP), insulin-like growth factor-binding protein 4 (IGFBP-4), inhibin α and glycoproteins, especially sulphated glycoprotein (Sgp-2), but inhibit the secretion of plasminogen activator and oestrogens in response to FSH (Rosselli and Skinner, 1992; Galdieri and Nistico, 1994; Guma and Bernard, 1994; Zhuang *et al.*, 1997; Gaemers *et al.*, 1998b; Bardi *et al.*, 1999; Sigillo *et al.*, 1999). Retinoids also act on the signalling pathways in Sertoli cells, reducing the expression of protein kinase C and of the androgen receptor, as well as the production of cAMP in response to FSH (Galdieri *et al.*, 1986; Galdieri and Nistico, 1994). In addition, retinoids stimulate the expression of certain transcription factors, such as c-jun and c-myb (Page *et al.*, 1996), and increase Sertoli cell metabolism, as they increase the expression of ornithine decarboxylase and cytochrome *c* oxidase (COX) (Gaemers *et al.*, 1998b; Klamt *et al.*, 2000). Retinoids are also involved in controlling Sertoli cell lipid metabolism, as shown by the accumulation of lipids in the tubules of RXR-β mutant mice (Kastner *et al.*, 1996). Curiously, lipid accumulation was also observed in the Sertoli cells of rats with hypervitaminosis A (Biswas and Deb, 1965).

Retinoids can also modulate their own signalling pathway in the testis, as they increase the expression of CRBP, RAR-α and RAR-β and prostaglandin D2 synthetase (Eskild *et al.*, 1988; Kim and Griswold, 1990; Faraonio *et al.*, 1993;

Gaemers *et al.*, 1997; Akmal *et al.,* 1998; Samy *et al.*, 2000). Prostaglandin D2 synthetase, which has a high affinity for retinoic acid and retinol, also serves as a retinoid transporter and is strongly expressed in the blood–testis barrier (Samy *et al.,* 2000).

The basement membrane, which is partly secreted by the peritubular cells, may alter the activity of Sertoli cells by modifying the availability of growth factors. Retinoids may affect the synthesis and deposition of extracellular matrix components through the peritubular cells, for instance by altering the synthesis and secretion of laminin and fibronectin (Ricci *et al.*, 1999).

The interactions between retinoids and FSH, the main regulatory hormone in Sertoli cell functions, are complex. Like retinoic acid, FSH stimulates the secretion of transferrin, ABP and inhibin α (De Jong, 1988; Skinner *et al.*, 1989) and the synthesis of retinyl esters (Guo *et al.*, 2001). However, retinoic acid inhibits the transduction pathway of FSH by blocking the production of cAMP as well as all the activities dependent on it, such as aromatase activity and the secretion of tissue-specific plasminogen activator. In return, FSH reduces the expression of RAR-α (Braun *et al.*, 2000).

Retinoids and spermatogenesis

In the testes of vitamin A-deficient rats, spermatogenesis was blocked at the A spermatogonia stage (Morales and Griswold, 1987) and retinoid supplementation retriggered spermatogenesis, which occurred concomitantly in all the tubules (Morales and Griswold, 1987). In such vitamin A-deficient rats, a high dose injection of retinoic acid strongly stimulated the proliferation of A spermatogonia and allowed their differentiation into B spermatogonia and then into spermatocytes, but not into spermatids (Van Pelt and de Rooij, 1991; Gaemers *et al.*, 1998c). Repeated injections of retinoic acid were necessary for spermatogonia to reach the spermatid stage successfully (Van Pelt and de Rooij, 1991). Huang and Marshall (1983) suggested that vitamin A deficiency may delay spermiation. Therefore, retinoids appear indispensable to the proliferation and differentiation of A spermatogonia, during their transition to round and elongated spermatids and spermiation.

RAR-α mutant mice display germinal epithelium degeneration very similar to that of vitamin A-deficient animals (Lufkin *et al.*, 1993). The location of RAR-α expression in the germ cells implies that retinoic acid exerts its effect on these cells via RAR-α (Akmal *et al.*, 1997; Dufour and Kim, 1999). RXR-β mutant mice are sterile, possibly because of deficient Sertoli cell functions (Kastner *et al.*, 1996), as a gradual accumulation of lipids was observed in Sertoli cells well before the usual degeneration of the germinal epithelium that occurs in old males. In addition, in the seminiferous tubules, RXR-β was expressed only in Sertoli cells (Kastner *et al.*, 1996; Dufour and Kim, 1999).

Retinoids can also be harmful at excessively high doses. Hypervitaminosis A in rats reduces the testicular mass, creates lesions in the seminiferous epithelium and perturbs the rhythm of spermatogenesis (Biswas and Deb, 1965; Lamano Carvalho *et al.*, 1978). As a result, the production of mature spermatozoa decreases and immature germ cells are extruded into the lumen of the seminiferous tubules. High doses of 13-cis retinoic acid, a very stable retinoid, block spermatogenesis completely (Sadek and Abdul-Mohsen, 1999).

Retinoids and steroidogenesis

In adult rats, vitamin A deficiency reduced basal testosterone secretion but testosterone secretion stimulated by exogenous LH remained similar to that of control rats (Appling and Chytil, 1981). This finding was supported by earlier reports of the atrophy of the accessory sex organs (prostate and seminal vesicles) and the female-type fine fur of vitamin A-deficient male rats (Thompson *et al.*, 1964). Hypervitaminosis A or injection of 13-cis retinoic acid reduced the volume of testicular interstitial tissue and the mass of the seminal vesicles, and degraded the cytoplasm of Leydig cells (Biswas and Deb, 1965; Lamano Carvalho *et al.*, 1978; Sadek and Abdul-Mohsen, 1999). These findings show that, in the same way as for spermatogenesis, both retinoid excess and retinoid deficiency are harmful to steroidogenesis.

The mechanism of action of retinoids is partly known. In adult rats, retinoids increased basal testosterone secretion in Leydig cell primary cultures but reduced this secretion when stimulated by LH (Chaudhary *et al.*, 1989). These apparently contradictory findings were accounted for by the results of the studies conducted on cell lines that originated from Leydig cells (Lefèvre *et al.*, 1994; Lee *et al.*, 1999) showing that retinol and retinoic acid reduce the expression of LH receptors and greatly increase the expression of certain enzymes involved in steroidogenesis, such as P450 C17α-hydroxylase–C17-20 lyase and steroidogenic acute regulatory protein. Thus, the predominant negative effect of retinoids on LH-stimulated testosterone secretion appears to be the reduction of the expression of LH receptors.

As with other specific functions, retinoids reduce basal testosterone production in fetal Leydig cells for a short period after testis differentiation (Livera *et al.*, 2000) and, thus, the action of retinoic acid on Leydig cells differs between the adult and the fetus (Habert *et al.*, 2001).

Conclusion

Retinoids are clearly involved in the regulation of testicular functions, and much progress has been made in understanding their mechanisms of action, although this understanding is far from complete. Research to date into the effects, mode of action and physiological involvement of retinoids in testicular functions has been conducted exclusively in rodents. It is important now to determine whether the therapeutic use of retinoids affects testicular functions in men.

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