Chapter 4
What compounds mediate communication within the testis? Where and how are male-associated hormones produced?
Integration of the hypothalamus, pituitary and testis

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Major functions of the testis include the synthesis and secretion of testosterone, and the generation of spermatozoa. We have known for decades that testosterone is essential for the initiation and quantitative maintenance of spermatogenesis. We now know that gene expression and cell-cell interactions of spermatogenesis are regulated by testosterone and, additionally, by FSH and paracrine factors. Testosterone is produced by the testicular Leydig cells in response to luteinizing hormone (LH) from the anterior pituitary. Follicle-stimulating hormone (FSH) also is produced by the anterior pituitary. Both LH and FSH are produced in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus. Testosterone and FSH function both independently and together to regulate the proliferation, maturation and function of Sertoli cells (Chapter 2). The Sertoli cells regulate and maintain the proliferation and differentiation of developing germ cells (Chapter 7). Thus, spermatogenesis regulation involves complex interactions of the hypothalamus, pituitary and testis.

Mechanisms of action of testosterone and FSH

LH, synthesized by and released from the anterior pituitary in response to GnRH from the hypothalamus, binds to G protein-coupled receptors on the cytoplasmic membrane of Leydig cells in the interstitial compartment of the testis, thus stimulating cAMP production. cAMP stimulates the translocation of cholesterol into the mitochondria, where it is metabolized by CYP11A1 of the inner mitochondrial membrane to pregnenolone. Pregnenolone is then

converted to testosterone by enzymes of the smooth endoplasmic reticulum. It is well established that testosterone is essential for the initiation, maintenance and restoration of quantitative spermatogenesis, but that the germ cells do not respond directly to androgens. Rather, the major target cells for testosterone within the testis are the androgen receptor - containing Sertoli cells. The very high intratesticular testosterone concentration that is present in seminiferous tubular fluid greatly exceeds the concentration needed to saturate the androgen receptors. However, bioavailable testosterone is reduced by its binding to androgen binding protein (ABP) produced by Sertoli cells, a mechanism that might reduce available testosterone considerably. Just how high the concentration of free testosterone must be for spermatogenesis is unclear, and likely differs in different organisms.

FSH acts through the Sertoli cells to regulate the proliferation and maturation of germ cells. FSH binds to plasma membrane-associated G-protein coupled receptors of Sertoli cells to stimulate their proliferation and the synthesis of secreted proteins, including transferrin and inhibin. Inhibin and testosterone are involved in feedback regulation of pituitary function. FSH also plays a significant role in the initiation of spermatogenesis at puberty. Its role in adult spermatogenesis of some species (e.g. rat) is uncertain, but it plays a highly significant role in spermatogenesis in man and non-human primates. In the absence of FSH action, the Sertoli cell number is decreased, and this has important effects on the quantity of sperm produced. FSH and testosterone together are involved in the regulation of germ cell maturation in part by regulating adhesion complexes between germ cells and Sertoli cells, and by their impact on germ cell entry into meiosis. Thus, although FSH and testosterone have independent mechanisms of action, with FSH acting through Sertoli cell membrane-bound, G-protein coupled receptors and testosterone through Sertoli cell androgen receptors, overlapping mechanisms in their modes of action exist. Although FSH is not a mandatory requirement for the completion of spermatogenesis or fertility in rodents, its deficiency nevertheless leads to significant reduction in sperm quantity.

Paracrine regulation of spermatogenesis

The seminiferous epithelium of mammals is highly organized to assure a constant supply of spermatozoa in very large numbers. Germ cell development involves maintaining a pool of stem spermatogonia through

renewal divisions, the differentiation and mitotic divisions of spermatogonia, meiosis, and spermiogenesis. As the germ cells divide and differentiate, they interact with the Sertoli cells and influence the expression of Sertoli cell genes, some of which encode growth factors that regulate germ cell differentiation, replication and survival. Thus, the Sertoli cells mediate the effects of testosterone and FSH, respond to paracrine and juxtacrine signals from the germ cells, and produce growth factors that are themselves involved in the maintenance and control of germ cell growth and differentiation.

The self-renewal and differentiation of the undifferentiated stem spermatogonial cells must be regulated so that the cells are not depleted. The Sertoli cell products kit ligand/stem cell factor (KL) and glial cell line- derived neurotrophic factor (GDNF), working together, are among the growth factors that play key roles in regulating the survival, renewal and differentiation of spermatogenic cells. Stem spermatogonia, the targets for GDNF, express the GDNF receptors Ret and GFRα1. In transgenic mice, loss of spermatogonial stem cells as a consequence of their differentiation occurs when GDNF is under-expressed, and repression of the differentiation of stem spermatogonia occurs in mice in which GDNF is over-expressed. Unlike GDNF, KL has many different target cells in the mature testis; its receptor, Kit, is expressed at high levels in differentiating spermatogonia, but also in meiotic cells. Further complicating matters is that the expressions of GDNF and KL themselves appear to be regulated both by paracrine signals from germ cells and by hormones.

A number of other growth factors are expressed in the testis, but their roles in sperm-atogenesis, if any, are uncertain. Insulin-like growth factor-1 (IGF-1) receptors are localized to germ cells, suggesting that IGF-1 may act on these cells. Transforming growth factor-α (TGF-α), TGF-β, and interleukin-1 (IL-1) are expressed, as are bone morphogenic protein 8a (BMP8a) and BMP8b. Basic fibroblast growth factor (bFGF), produced by pachytene spermatocytes, is required for the in vitro replication of stem spermatogonia in some, but not all, strains of mice.

The organization of the seminiferous epithelium is initiated and maintained in part by the actions of retinoic acid (RA). RA is initially made by Sertoli cells and acts on undifferentiated spermatogonia to stimulate them to irreversibly enter into differentiation and ultimately to form spermatozoa in a series of timed molecular events. The time required to form spermatozoa is species-specific, ranging from 30 to over 70 days. To assure a constant supply of

sperm, the action of RA to stimulate undifferentiated spermatogonia is staggered and progressive along the seminiferous tubules in the form of RA pulses. As the differentiating spermatogonia progress to sperm, they move towards the lumen and overlap the germ cells developing from previous pulses. Well-defined groups of germ cells, termed stages (also known as cell associations), appear along the tubules. The stages are morphologically distinct and can be identified in histological testis tubule cross sections. The action of RA on undifferentiated spermatogonia results in the induction of key genes such as Stra8 and Kit that are necessary for the completion of meiosis, and the decreased transcription of genes such as Pou5f1, Nanos2, Lin28a, and Zbtb16 that characterize the undifferentiated spermatogonia. The enzymes involved in the synthesis of, and signaling by, RA are found in mice and in the human testis as well, but there is little information available regarding the effect of RA on human male germ cells. Clearly there are large gaps in our knowledge with respect to RA regulation of human spermatogenesis. Nonetheless, the retinoic acid synthetic pathway has been proposed both as a possible origin of some infertility and as a potential male contraceptive target.

Suggested reading

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