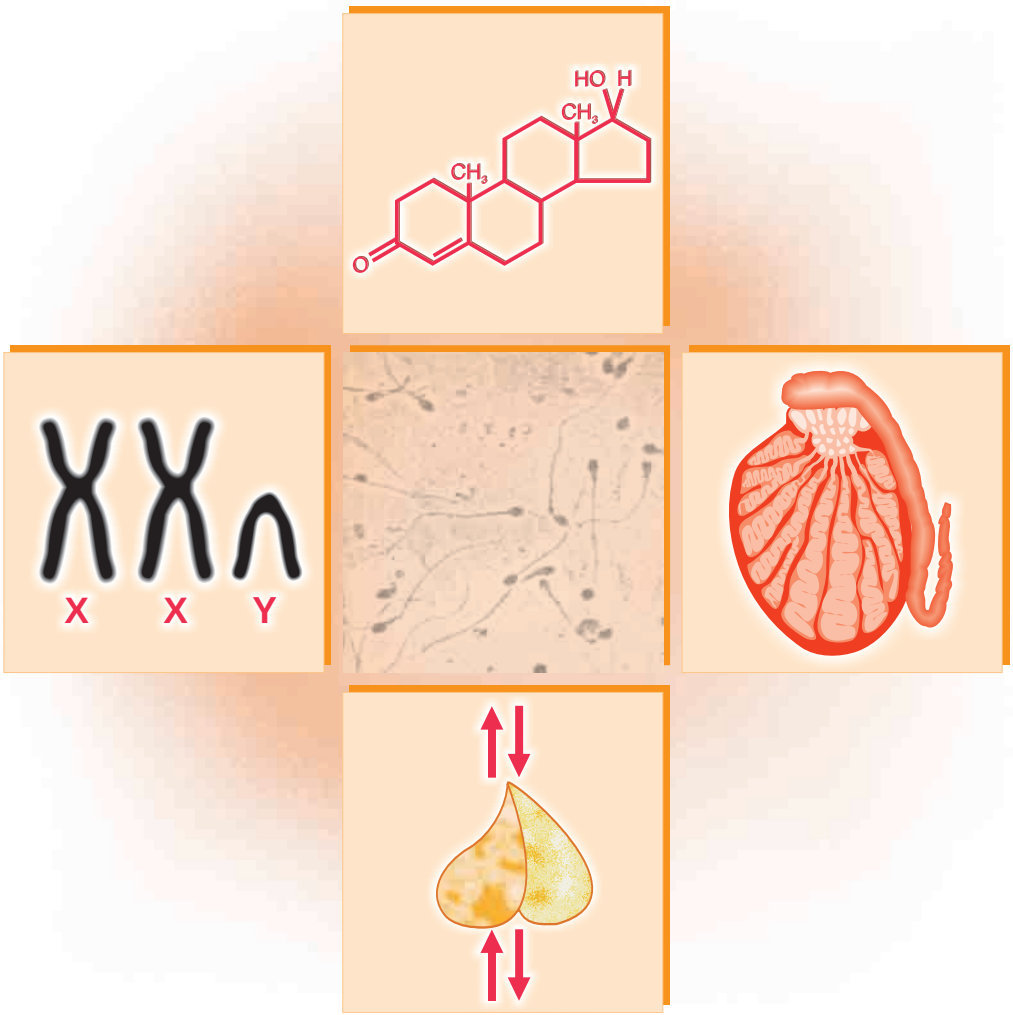
Male hypogonadism

4th edition

Michael Zitzmann
Friedrich Jockenhövel
Markus Schubert



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Male hypogonadism

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UNI-MED Verlag AG, one of the leading medical publishing companies in Germany, presents its highly successful series of scientific textbooks, covering all medical subjects. The authors are specialists in their fields and present the topics precisely, comprehensively, and with the facility of quick reference in mind. The books will be most useful for all doctors who wish to keep up to date with the latest developments in medicine.

Foreword 4th edition

One of the most frequent, but also most underdiagnosed, endocrinopathies is male hypogonadism (testosterone deficiency). Male hypogonadism presents with a vast clinical picture that is most often associated with typical symptoms, such as disturbances of mood as well as sexual functions. Furtheron, a decrease in muscle mass and strength, an accumulation of body fat and osteopenia/osteoporosis are frequently observed. There are indications that insulin sensitivity is attenuated by androgen deficiency. Especially in older men, symptoms of androgen deficiency may exhibit a differential profile due to accompanying other chronic illnesses. Restoring serum testosterone levels by replacement therapy can markedly mitigate, if not totally relieve, the clinical picture of hypogonadism. Nevertheless, in-depth understanding of the underlying pathologies and diagnostical procedures is essential and we are happy to provide the reader with these clinical tools.

New treatment modalities have been introduced during the recent years, which include short-acting transdermal modalities as well as the long-acting depots of testosterone.

This book gives detailed and up-to-date information regarding the various modern methods of diagnosis of andrological pathologies, initiation and surveillance of testosterone substitution therapy and/or other hormone substitution modalities.

The book pays special attention to the ageing patients and their specific needs and we are happy to provide the reader with a vast range of case discussions, to facilitate the transfer of theoretical and recent knowledge into daily clinical practice.

Michael Zitzmann

Foreword 3rd edition

The aim of this book is to provide cutting edge knowledge on the diagnostics and therapeutic procedure for men with impaired testicular endocrine function.

The book provides in depth background information on anatomy and physiology of endocrine testicular function. Where necessary, the most recent developments in molecular biology are covered. The clinical presentation of male hypogonadism is described in detail, as well as all procedures necessary for a precise diagnosis.

An extensive chapter deals with all aspects of androgen substitution therapy and the misuse of androgens. Most recent and future developments, such as transdermal applications including testosterone gel and the long-acting intramuscular testosterone formulations are described in depth. For each formulation and application system the individual advantages and disadvantages are discussed.

Androgen deficiency in the aging male is covered by a separate chapter. This recognizes the importance of this growing field of interest and the specific diagnostic and therapeutic aspects of this subject. Also, a specialised chapter covers the subject of estrogen deficiency and therapy with estrogens in men.

We are most grateful to the staff from UNI-MED Verlag for their excellent support.

Friedrich Jockenhövel
Markus Schubert

Preface

This excellent text on male hypogonadism provides a comprehensive yet focused view of testicular function and hormone related abnormalities involving the male reproductive system. Friedrich Jockenhövel has managed to provide the background that is so often demanded by the specialist and presents the information in a fashion that is easily understandable by the generalist.

There has been remarkable progress in male reproductive medicine in the past several decades. The book demonstrates how the practitioner distinguishes central (hypothalamic or pituitary) from primary testicular causes of testosterone deficiency and provides a differential diagnostic approach to congenital and acquired forms of hypogonadism. The complex mechanisms of action of androgens at multiple target organs have provided the groundwork for understanding the benefits and risk of testosterone therapy. A perception of male hormones as essential for male sexuality has now been greatly expanded to a broader understanding of the metabolic aspects of circulating androgens. This text describes the role of androgens on such diverse organs as liver, bone, muscle, fat and the central nervous system. We have come to know testosterone as not only a hormone, but a pre-hormone serving as the precursor to metabolically active hormones (dihydrotestosterone and estradiol). Thus, testosterone affects may be mediated through both androgen and estrogen receptors. Androgen deficiency presents differently at different ages. This book characterizes testosterone deficiency occurring during fetal development at puberty and adulthood. We have come to recognize that testosterone levels in the blood fall progressively with age and that there is a significant percentage of the older male population whose circulating testosterone levels are considerably lower than that of their young healthy male counterparts. We have also come to understand the effects of the increasing sex hormone binding globulin concentration and its impact on decreasing free and bioavailable testosterone as men get older. The next decade will undoubtedly reveal the relative benefits and risks of treating older males with androgen deficiency with therapeutic doses of testosterone. The recognition of significant bone disease in men with aromatase and estrogen receptor deficiency has lead us to appreciate the important role of estrogens in normal male physiology. This recognition will influence the way we look at new synthetic androgens with selective metabolic actions. Multiple new androgenic preparations have become available giving greater flexibility to the physician in treating hypogonadal disorders. At present, transdermal preparations of testosterone have captured much of the market in the United States and Europe, and the future will reveal new safe oral testosterone delivery systems and long acting depo preparations requiring only three to four injections per year. In the next several years, large numbers of synthetic androgen receptor modulators will also become available. These drugs will have selective effects on various target organs and allow focused therapy with androgens for specific indications. This text also describes condition of estrogen deficiency in the male, originally appreciated by the careful scrutiny of a few individuals who had estrogen receptor defects and/or the inability to convert androgens to estrogens due to aromatase deficiencies. In the latter sections of the book, Dr. Jockenhövel's case management format is ideally suited for the clinician in his/her strategic approach to the diagnosis and management of the androgen deficient patient.

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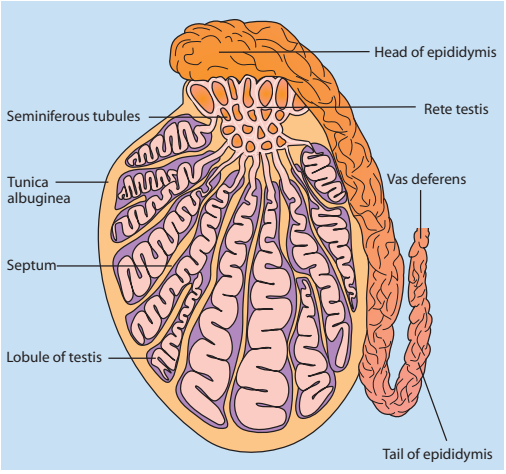
1. Anatomy and physiology of the testis

The dual function of the testis as an endocrine (hormone-producing) and exocrine (sperm-producing) gland affords it a special position among the endocrine organs. This is reflected in the anatomy and physiology of the testis. The testicular parenchyma, which is surrounded by a solid capsule (tunica albuginea), consists of seminiferous tubules in which gametes are produced (spermatogenesis). Septa of connective tissue divide the testis into 200-300 lobules; these combine in the rete testis. Each lobule contains two to three seminiferous tubules which average 50 cm in length and are very convoluted. Each testis contains altogether 600-900 seminiferous tubules, approximately 350 m in total length. This explains the enormous reproductive capacity of the human male, who can produce approximately 10-20 million gametes per day. In the interstices between the seminiferous tubules are the steroid-producing Leydig cells, as well as the necessary blood and lymph vessels and nerves. Approximately 85-90% of the volume of the testis is taken up by the seminiferous tubules, and only 10-15% by the interstices (☞ Figure 1.1)[209].



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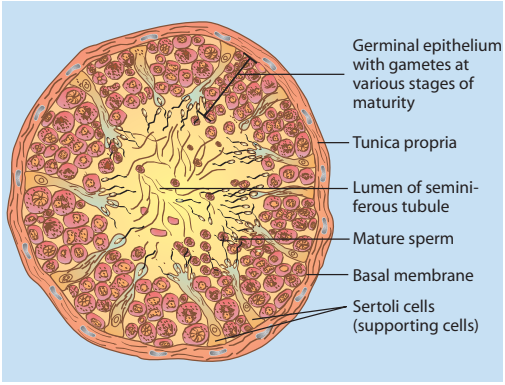

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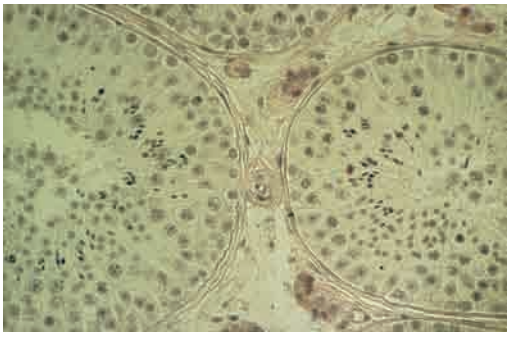

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Figure 1.1: **(a)** Macroscopic cross-section through the testis and epididymis; **(b)** microscopic section from (a) showing seminiferous tubules; **(c)** histological cross section showing cut surfaces of three seminiferous tubules with intact spermatogenesis. Between the tubules is the interstice containing Leydig cells and a blood vessel (HE stain, 40× magnification).

1.1. Hypothalamic-hypophyseal regulation

The hypothalamus controls the function of the testis by means of the pituitary hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH) (☞ Figure 1.2). In the hypothalamus, gonadotropin releasing hormone (GnRH) is produced under the stimulating and inhibiting influence of neurotransmitters. GnRH promotes the production and release of the gonadotropins LH and FSH in the hypophysis.

In humans, GnRH neurons are initially laid down in the region of the cribriform plate, and adhesion

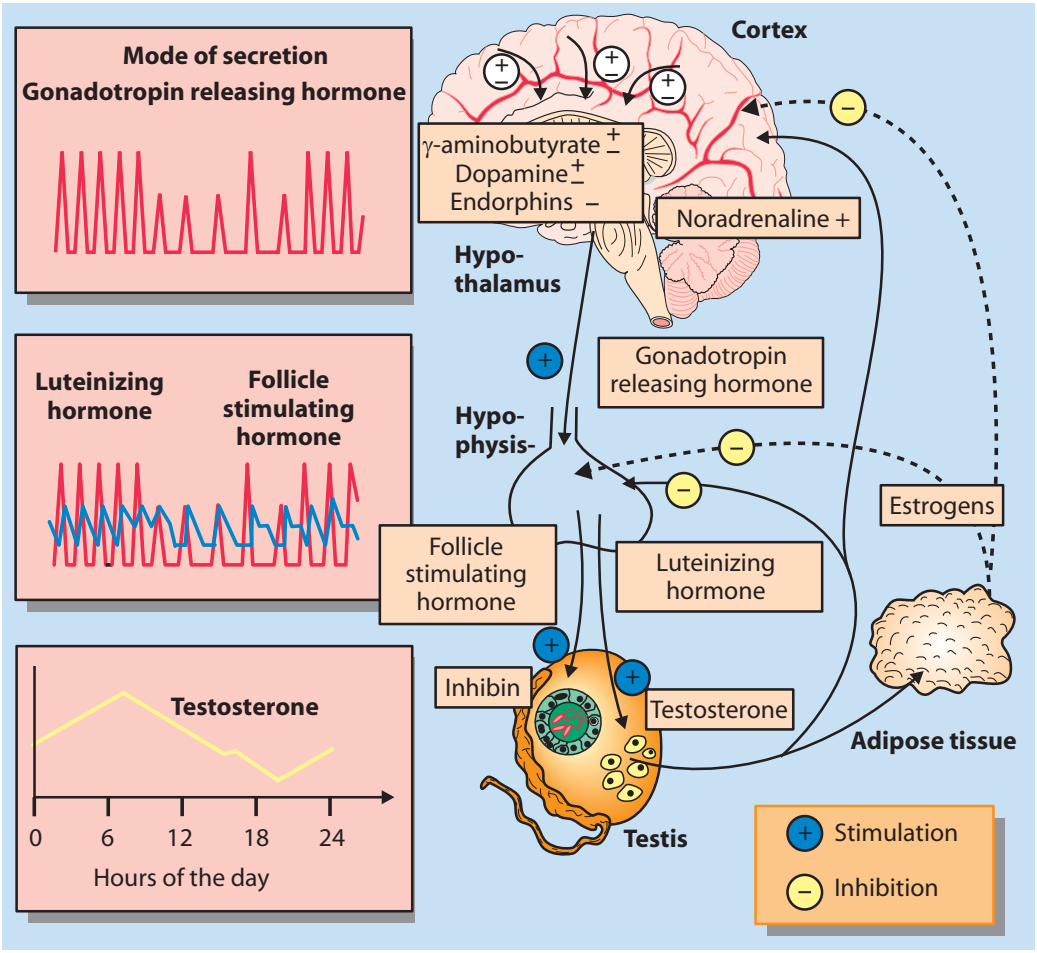

Figure 1.2: Regulation of testicular function and secretion pattern of the hormones involved. Gonadotropin releasing hormone (GnRH) is secreted from the hypothalamus under the influence of neurotransmitters (γ-aminobutyrate, catecholamines, endorphins) and from the cortex in a pulsatile fashion, and stimulates production of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the hypophysis. LH (shown in red) follows the secretion profile of GnRH very closely, whereas FSH (blue) does not display such clear pulsatility owing to its longer half-life. LH stimulates testosterone biosynthesis in the Leydig cells. Testosterone exerts a direct, and after metabolism to estrogens an indirect, negative feedback effect on the secretion of GnRH and gonadotropins. Together with testosterone, FSH stimulates the production of peptides such as inhibin, which in turn exerts a negative feedback effect on FSH.

molecules cause them to migrate to the arcuate nucleus and mediobasal brain centers of the hypothalamus. Already in the fetus GnRH is produced under the control of neurotransmitters. Dopamine, serotonin and GABA exert inhibitory effects and NPY stimulating effects.

GnRH is formed in the arcuate nucleus and the preoptic region of the hypothalamus. Its axons innervate the median eminence and secrete GnRH into the portal system of the hypophysis. GnRH is initially produced in the form of prepro GnRH. Prepro GnRH is encoded by a gene on chromosome 8 (8p21-p11.2). The decapeptide GnRH arises by post-translational enzymatic cleavage of the leading 23 amino acids ('pre' portion) and of the supporting 56 amino acids ('pro' portion = GnRH-associated peptide, GAP). The enzyme prohormone convertase-1 is responsible for this.

GnRH is secreted in regular pulses, with peaks every 90-120 min. This is due to the intrinsic ability of GnRH neurons to secrete episodically. This basic rhythm is modulated by numerous neurotransmitters. Pulse amplitude and pulse frequency affect the amount of LH and FSH secreted by the hypophysis. α-Adrenergic impulses have a stimulatory action, and β-adrenergic and dopamine impulses an inhibitory action on GnRH secretion. Endorphins display cycle-dependent effects in women but have a predominantly inhibitory action in men. Testosterone and progesterone slow down this pulse rate, presumably mediated by β-endorphins. The adverse effect of stress on reproductive function is well known. Several factors are involved: corticotropin releasing hormone (CRH) inhibits GnRH secretion through direct neuronal contact between the paraventricular nucleus and preoptic region. The level of prolactin, which is often raised in stress, further reduces the GnRH pulse rate. In addition, central cytokines, such as the inhibitory interleukin 1, also appear to play a role. Recent findings indicate that leptin, presumably indirectly via neuropeptide Y (NPY), intervenes in the feedback mechanism. Therefore, the administration of leptin to leptin-deficient obese infertile rats not only reduces weight but also restores fertility. Leptin is also likely to be involved in the initiation of puberty. GnRH has a half-life of 5-10 min but cannot be measured owing to its low systemic concentrations.

Furtheron, the discovery of kisspeptin as key central regulator of GnRH secretion has led to a new level of understanding of the neuroendocrine regulation of human reproduction. The related discovery of the kisspeptin-neurokinin B-dynorphin (KNDy) pathway in the last decade has further strengthened the understanding of the modulation of GnRH secretion by endocrine, metabolic and environmental inputs. Kisspeptin is a principal regulator of the secretion of gonadotrophins, and through this key role it is critical for the onset of puberty, the regulation of sex steroid-mediated feedback and the control of adult fertility. Although there sexual dimorphism exists, both neuroanatomically and functionally, these functions are apparent in both men and women. Kisspeptin acts upstream of GnRH and, following paracrine stimulatory and inhibitory inputs from neurokinin B and dynorphin (KNDy neuropeptides), signals directly to GnRH neurones to control pulsatile GnRH release. When administered to humans in different isoforms, routes and doses, kisspeptin robustly stimulates LH secretion and LH pulse frequency. Manipulation of the KNDy system is currently the focus of translational research with the possibility of future clinical application to regulate LH pulsatility, increasing gonadal sex steroid secretion in reproductive disorders characterized by decreased LH pulsatility, including hypothalamic amenorrhoea and hypogonadotropic hypogonadism[360a].

In the anterior lobe of the pituitary, GnRH binds to specific receptor (typical G-protein-bound receptors with seven transmembranous loops) on the gonadotropic cells and initiates the gene expression of α- and β-chains of FSH and LH and their secretion by induction of inositol-1,4,5,-triphosphate, mobilization of intracellular calcium and increased calcium influx. Whereas androgens reduce the number of GnRH receptors, the influence of estrogens is dependent on the concentration of estrogen, the dynamics of the concentration change and other factors.

The pulsatility of GnRH is essential to its gonadotropin-stimulating effect. Continuous administration of GnRH causes gonadotropin production to cease completely owing to down regulation of the GnRH receptor. This effect is utilized therapeutically in the administration of slow-acting GnRH analogs in conditions which are dependent on sex steroids (e.g. endometriosis, carcinoma of the prostate, precocious puberty). In patients with hypothalamic-induced hypogonadism, e.g. Kallmann's syndrome, correct pulsatile administration of GnRH can establish normal LH and FSH concentrations and hence normal gonadal function.

The polypeptides LH and FSH are large glycoproteins like thyroid stimulating hormone (or thyrotropin, TSH) and human chorionic gonadotropin (hCG). A common characteristic is the noncovalent binding of two peptide chains (α and β) to a heterodimer; the α-chain is identical in all four hormones, and therefore the biological effect is mediated by the β-chain. The α- and β-chains are encoded by genes on different chromosomes. The α-chain is encoded by a gene on chromosome 6 (6q12.21), FSH-β on chromosome 11 (11p13) and

LH-β and hCG-β on chromosome 19 (19q13.32). Isolated subunits and homodimers show no biological activity. The strong similarity of the basic structure, the high homology of the amino acid sequence and the organizational structure of the genes make the phylogenetic origin of all glycoproteins by gene duplication from a common precursor gene very likely. In phylogenesis, the gonadotropins developed later than TSH; they show more homology among themselves than with TSH. Gene duplication of LH and hCG occurs even later, so these hormones are almost identical and have the same biological action. However, hCG has a significantly longer half-life. Owing to gene duplication, which is still young in terms of evolutionary development, the genes for the β-chains of LH and hCG lie in a gene complex with six gene duplications of hCG-β and one gene for LH-β on chromosome 19 (19q13.32).

Moreover, a common feature of all chains of the glycoprotein hormone family is co-translational glycosylation. This means that, at certain points on the peptide chain, carbohydrates are bound to the amino acid asparagine. The structure is usually $Glc_{1\text{-}3}$(α-mannose 4-6-mannose $\beta_{1\text{-}4}$Glc)nAC $\beta_{1\text{-}4}$Glc-NAc-Asn. These carbohydrates are then further modified by the removal of glucose and mannose portions and the incorporation of other oligosaccharides (e.g. fucose, galactose and sialic acid), so that even more complex carbohydrate side-chains arise on the amino acid chain. This process is not always identical. Therefore, minimal differences in carbohydrate side-chains cause different forms of glycoproteins to be secreted, which may be separated from each other by special procedures (e.g. isoelectric focusing). Hence, seven different species of LH are found in man which differ only in their carbohydrate composition. The carbohydrate side-chains influence the tertiary structure, binding of α- and β-chains, half-life in the systemic circulation, binding to specific receptors and also intracellular signal transduction after receptor binding in the target cell. In particular, a high concentration of sialic acid prevents metabolism of glycoproteins in the liver and kidneys, and thus prolongs the half-life and biological effect. In TSH and FSH, the carbohydrate side-chains terminate with sialic acid, which explains the longer half-life compared with LH. Thus, LH has a half-life of only 20 min, whereas that of FSH is 3 h and hCG 5 h[181].

LH binds to specific receptors on the surface of the Leydig cells and, mediated by cyclic adenosine monophosphate (cAMP), causes an increase in intracellular cholesterol and increased gene expression of enzymes of steroid production, in particular the key enzyme 20,22-desmolase. This initiates biosynthesis of testosterone with cleavage of the side-chain from cholesterol. The feedback control of LH production in man occurs via testosterone and its metabolite estradiol. Testosterone has an inhibitory effect on the neurons producing GnRH, and exerts only a slight suppressant effect on hypophyseal LH production. In contrast, estradiol has an inhibitory action on the hypophysis and hypothalamus.

FSH binds to receptors on the Sertoli cells and promotes spermatogenesis in a manner that is as yet unexplained. Among other things, the activity of the enzyme aromatase, which converts androgens to estrogens, is stimulated in the Sertoli cells. In addition to a number of other proteins, the hormones inhibin and activin are formed in the Sertoli cells under the influence of FSH. Inhibin is a heterodimer of the α and β subunits; there are two β-variants (β_a and β_b). Hetero- and homodimers of the β-chains are called activin A (β_a-β_b) and activin B (β_b-β_b). Inhibin is an important component in the feedback system controlling FSH secretion. In isolated functional disturbances of the Sertoli cells (e.g. Sertoli-cell-only syndrome or following radiotherapy or chemotherapy), inhibin deficiency is indicated by a sharp rise in FSH while LH remains at normal levels. The physiological significance of FSH-stimulating activins has not been conclusively explained; however, they appear to be less important. Testosterone and estrogens also exert a negative feedback effect on FSH via their effect on GnRH.

Gonadotropins, on the other hand, promote testicular function directly. LH binds to specific receptors on the Leydig cells and, in a cAMP-mediated process, stimulates the production of enzymes which initiate biosynthesis of testosterone with cleavage of the side-chain from cholesterol. FSH binds to receptors on the surface of the Sertoli cells and the spermatogonia and, together with testosterone, promotes spermatogenesis. Like the

GnRH receptor, gonadotropin receptors are G-protein-bound receptors with seven transmembranous loops. The genes of the LH and FSH receptors both lie on chromosome 2.

1.2. Spermatogenesis

Spermatogenesis is a complicated process of mitotic and meiotic cell divisions in which haploid spermatids are formed from diploid spermatogonia via several intermediate stages (☞ Figure 1.3). Spermatids are transformed into flagellated spermatozoa by a process of metamorphosis, called spermiogenesis. After spermiogenesis is complete, the spermatozoa are released from the germinal epithelium (spermiation) and flow into the epididymis with the fluid from the tubules, where they await ejaculation. The entire process of spermatogenesis and spermiogenesis takes 72 days in humans. Until spermiation, the gametes are dependent on the nursing Sertoli cells, which extend from the basal membrane of the seminiferous tubules deep into the lumen; the Sertoli cells secrete electrolytes and fluid under the influence of FSH and testosterone.

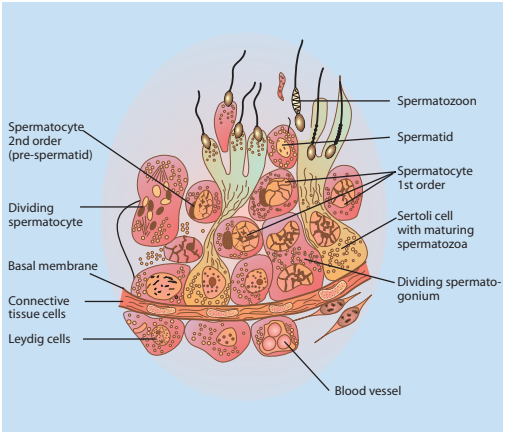

Figure 1.3: Section through a seminiferous tubule. The gametes are embedded between the Sertoli cells and migrate during development from the basal membrane to the lumen, where the spermatozoa are released.

After puberty, spermatogenesis can be maintained without FSH solely by sufficiently high intratesticular concentrations of testosterone. The exact function of the Sertoli cells and the mechanisms behind the paracrine interaction with gametes are unknown; however, the Sertoli cells are imperative to spermatogenesis. At their base, Sertoli cells form 'tight junctions' with one another, which seal the intracellular gap and thus form a blood-testis barrier. Like the blood-brain barrier, the blood-testis barrier is impermeable to macromolecules. The lumen of the seminiferous tubules is divided into two compartments in parallel with the basal membrane: in the basal region in 'front' of the blood-testis barrier are the spermatogonia, and 'behind' the blood-testis barrier all the more advanced stages of spermatogenesis take place. Therefore, the composition of the fluid in the tubules (adluminal compartment) is exclusively determined by secretion from the Sertoli cells, and its composition differs from that of serum or interstitial fluid (e.g. electrolyte, protein, glucose, amino acid content).

In the fetus, the Sertoli cells produce a polypeptide called anti-Müllerian hormone (AMH), which prevents the formation of internal female genitalia (uterus, Fallopian tubes) from the Müllerian duct during sexual differentiation. AMH can be detected in the serum until puberty. The postnatal significance of AMH is unclear. After puberty the production of AMH stops. The Sertoli cells also produce the hormones inhibin and activin. These peptides consist of two polypeptide chains linked together by disulfide bridges; they exert both a positive and a negative feedback effect on FSH[95, 176,319,407].

1.3. Testosterone and androgen effect

1.3.1. Testosterone biosynthesis

While the sequence of steroid synthesis including all intermediate stages has been known for decades, new and detailed findings have recently been made with the aid of molecular biology techniques. Hence, the genes of all enzymes required for testosterone biosynthesis have been identified, cloned and expressed in transformed cells. This allows a completely new insight into the regulation of steroidogenesis from a molecular viewpoint, and has shed light on a number of rare disorders caused by enzyme deficiencies in steroid synthesis.

Testosterone is the most important steroid produced by the testis; 5-7 mg (!) testosterone are produced each day by the Leydig cells of an adult man.

Like all steroid-producing cells, the Leydig cells have a large endoplasmic reticulum and numerous mitochondria. They also typically contain Reinke crystalloids, but the function of these is unknown.

The parent substance of testosterone biosynthesis is cholesterol, which is mainly synthesized by the Leydig cells; only a small amount of cholesterol is taken from the circulating blood[435]. Cholesterol is stored in form of esters in fat vacuoles in the Leydig cells until further processing. Through a total of five enzymatic stages, cholesterol, which contains 27 carbon atoms, is hydrolyzed to C19-testosterone (☞ Figure 1.4).

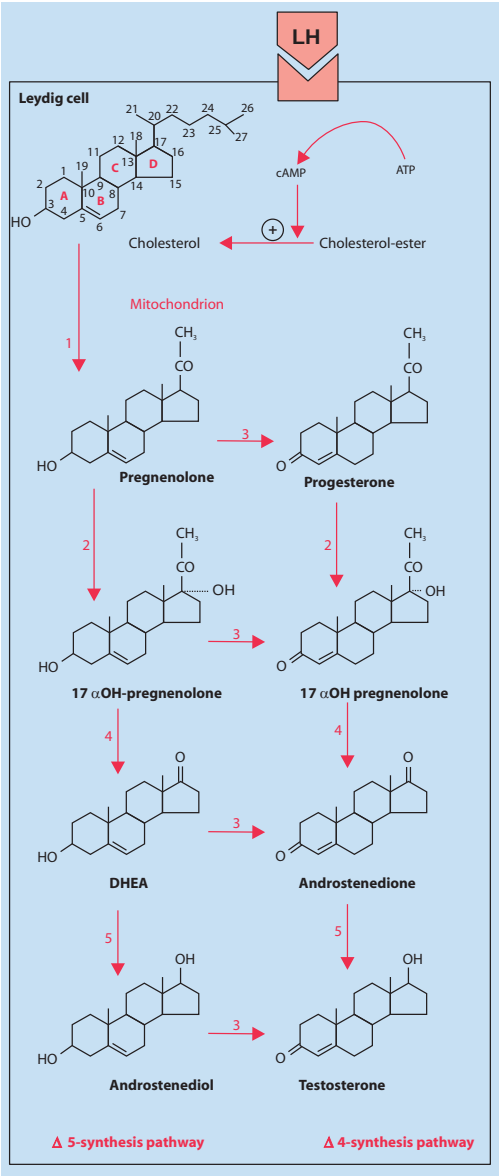

Figure 1.4: Biosynthesis of testosterone in Leydig cells. 1 = 20,22-desmolase; 2 = 17α-hydroxylase; 3 = 3β-hydroxysteroid dehydrogenase; 4 = 17,20-desmolase; 5 = 17β-hydroxysteroid dehydrogenase.

The most significant and rate limiting step in the enzymatic cascade is the conversion of cholesterol to pregnenolone. This takes place on the inner side of the membrane of the mitochondria, where the membrane's own enzyme cytochrome P450scc (side-chain cleavage) catalyzes three consecutive stages: first hydroxylation on atom C20, then on

atom C22 and thereafter cleavage between C20 and C22, giving pregnenolone and isocaproic acid. Cytochrome P450ssc, also known as 20,22-desmolase, is the crucial enzyme in all the steroid-producing tissues (adrenal gland, ovary) and is encoded by a gene on chromosome 15. Pregnenolone is the parent substance of all biologically active steroid hormones (corticosteroids, mineralocorticoids, gestagens, estrogens, androgens) and leaves the mitochondria by diffusion. It undergoes further processing in the endoplasmic reticulum. There are two pathways available: these are called the Δ4- or Δ5-synthesis pathways, depending on whether the double bond is located in ring A or ring B. The Δ5-synthesis pathway is preferred in man. Accordingly, hydroxylation frequently occurs first in position C17 by the action of cytochrome P450c17 (17α-hydroxylase), to 17α-hydroxypregnenolone. The weak androgens dehydroepiandrosterone (DHEA) and androstenediol are produced by the enzymes 17,20-desmolase and 17β-hydroxysteroid dehydrogenase. A further important step is the conversion of the less biologically active Δ5-steroids 17α-pregnenolone, DHEA and androstenediol to the corresponding more effective Δ4 steroids 17α-progesterone, androstenedione and testosterone. This step is catalyzed by the enzyme 3β-hydroxysteroid dehydrogenase, and comprises oxidation of the 3β-hydroxyl group to ketone with subsequent transfer of the double bond from C5-C6 on ring B to C4-C5 on ring A (Δ5-Δ4-isomerization). The majority of testosterone produced in this way is immediately released into the blood, and not stored within the testes. Most testosterone is transported by the spermatic vein; a small amount of testosterone is transported in the lymphatic system.

Testosterone biosynthesis is regulated by LH which influences the most critical step of synthesis, the conversion of cholesterol to pregnenolone, via two mechanisms:

- cAMP-mediated stimulation of the synthesis and activity of cytochrome P450ssc
- Protein kinase-C-mediated increase in the production of cholesterol by activation of hydrolase, an ester of cholesterol

Mobilization of cholesterol from the fat vacuoles renders more substrate available to the mitochondria for conversion to pregnenolone. Recent studies indicate that an as yet unidentified factor in the Sertoli cells, which is in turn produced under the influence of FSH, promotes steroid biosynthesis.

1.3.2. Transport of testosterone in the blood

As testosterone is lipophilic, it passes easily through membranes and leaves the Leydig cells by diffusion. In the blood, 98% of testosterone is bound to transport proteins, and only 2% is free and hence biologically active. Approximately 60% of the circulating testosterone is bound with high affinity to the β-globulin sex hormone binding globulin (SHBG), and 38% is loosely bound and transported by albumin. SHBG is a large glycoprotein (92.5 kDa) and is encoded by a gene on chromosome 17 in the immediate vicinity of the tumor-suppressant gene p53 (17p13.1). SHBG circulates in the serum as a homodimer, and carries two binding sites for sex steroids. Circulating SHBG is produced by the liver; however, SHBG is also locally produced by other tissues, e.g. the prostate and mammary gland[202]. SHBG shows a higher affinity for testosterone than for estradiol. Thus, increased production of SHBG by the liver causes a shift in the ratio of testosterone to estradiol by reducing the amount of free testosterone. As androgens reduce SHBG production, men have lower serum concentrations of SHBG than women. On the other hand, serum SHBG may be raised in men with testosterone deficiency. Other factors influencing SHBG production are listed in Table 1.1[323].

Stimulation of SHBG production	Inhibition of SHBG production
• Estrogen intake	• Androgen therapy
• Androgen deficiency	• Overweight
• Growth hormone deficiency	• Acromegaly
• Hyperthyroidism	• Hypothyroidism
• Hepatitis	• Nephrotic syndrome
• Liver cirrhosis	• Corticosteroids
• Phenytoin	• Hyperinsulinism
	• Gestagens

Table 1.1: Factors influencing sex hormone binding globulin (SHBG) serum concentrations.

Recent findings indicate that, in addition to transporting sex steroids, SHBG also has other func-

tions. It may bind to specific, recently discovered, SHBG receptors on cell membranes, and hence possesses the ability to bind to testosterone or estradiol simultaneously. When a sex steroid binds to SHBG, the SHBG receptor complex is obviously activated by conformational changes, triggering an effect inside the cell. This may be the mechanism of the rapid non-genomic effects of testosterone and estradiol which occurs within minutes, and therefore may not follow the vival androgen and estrogen receptor-mediated pathways. It is unclear whether SHBG exerts autonomous effects equivalent to those of a hormone[157,324].

1.3.3. Metabolism of testosterone

Free testosterone diffuses passively into the target cells where it may be metabolized, depending on enzyme availability, to 5α-dihydrotestosterone (DHT) or 17β-estradiol (☞ Figure 1.5). In humans, 2 isoenzymes of 5α-reductase, which show large homology, are responsible for conversion to DHT. Type I 5α-reductase is encoded by a gene on chromosome 5. It is found mainly in the skin and liver. A gene on chromosome 2 carries the information for type II 5α-reductase, which is predominantly found in the prostate, adrenal gland, seminal vesicle, genital skin, hair follicles and cerebral cortex. Approximately 80% of circulating DHT is produced by the peripheral conversion of testosterone, and 20% is secreted directly by the testis.

DHT and testosterone bind to the same androgen receptor, although DHT has a more than ten-fold greater affinity for the receptor and its dissociation from it is slower. Therefore, it is the more potent androgen. In physiological concentrations, the effects of testosterone and DHT complement one another. Therefore, both hormones are required for normal sexual development and virilization in puberty.

It is not clear whether DHT also induces effects which testosterone cannot, even in supraphysiological concentrations[99,136].

Furthermore, approximately 30 µg estradiol are produced per day by extratesticular aromatization of testosterone and androstenedione. Adipose tissue, bone cells and the prostate are rich in aromatase activity and can also produce estradiol. In addition, approximately 10 µg estradiol are directly produced by the Leydig cells.

Hence, testosterone is the prohormone for DHT and estradiol.

The complete spectrum of action of testosterone incorporates effects which are indirectly induced by conversion to DHT and estradiol (☞ Figure 1.6).

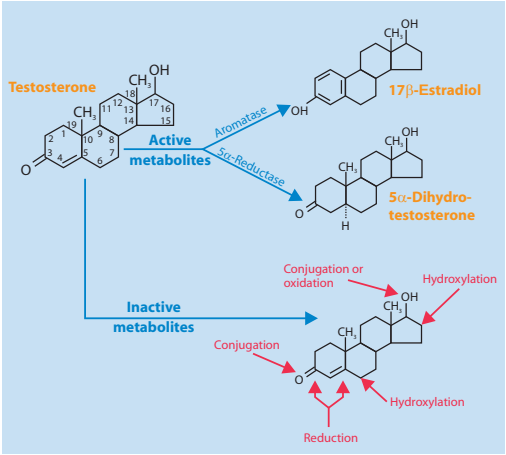

Figure 1.5: Metabolism of testosterone. In target organs, testosterone may be metabolized to 5α-dihydrotestosterone (DHT) or 17β-estradiol, depending on enzyme availability. Androgens are inactivated in the liver by oxidation, reduction or hydroxylation at different positions on the molecule.

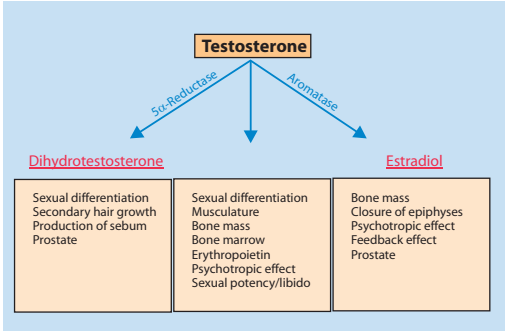

Figure 1.6: The effects of testosterone are either mediated directly (middle) or after metabolism to dihydrotestosterone (DHT) (left) or estradiol (right).

Testosterone and DHT are catabolized in the liver by two steps. First, the polarity of testosterone is increased by oxidation, reduction or hydroxylation. The initial and rate limiting step is reduction of the

C4,5 double bond (☞ Figure 1.5). In the second step, the water solubility of the lipophilic steroid is facilitated by conjugation with glucuronic acid or sulfation on C3 or C17 of the androgen molecule. Elimination takes place via the urine as 17-ketosteroid and sulfate (e.g. androsterone, etiocholanolone, epiandrosterone, epitestosterone)[333]. Despite being bound to transport proteins in the blood, elimination of testosterone from the serum by the liver is very efficient - the half-life of free testosterone in the blood is only about 10 min.

1.3.4. Structure and function of the androgen receptor

The androgen receptor belongs to the family of steroid and thyroid hormone receptors, and is encoded by eight exons of a gene near the centromere on the long arm of the X chromosome (Xq11-12). The androgen receptor is a polypeptide of 910 amino acids with a molecular weight of 98.5 kDa[59].

Like the other steroid and thyroid hormone receptors, the androgen receptor is a DNA binding protein. Its activity is ligand-dependent and transcription-regulating. The androgen receptor carries three domains with different functions (☞ Figure 1.7).

The aminoterminal segment (exon A) inside steroid hormone receptors is very variable. It appears to affect transcription and to maintain the tertiary and quaternary structure of the androgen receptor.

The centrally located hydrophilic DNA-binding domains (exons B and C) carry two 'zinc' fingers, which bind to specific sections of DNA in or next to

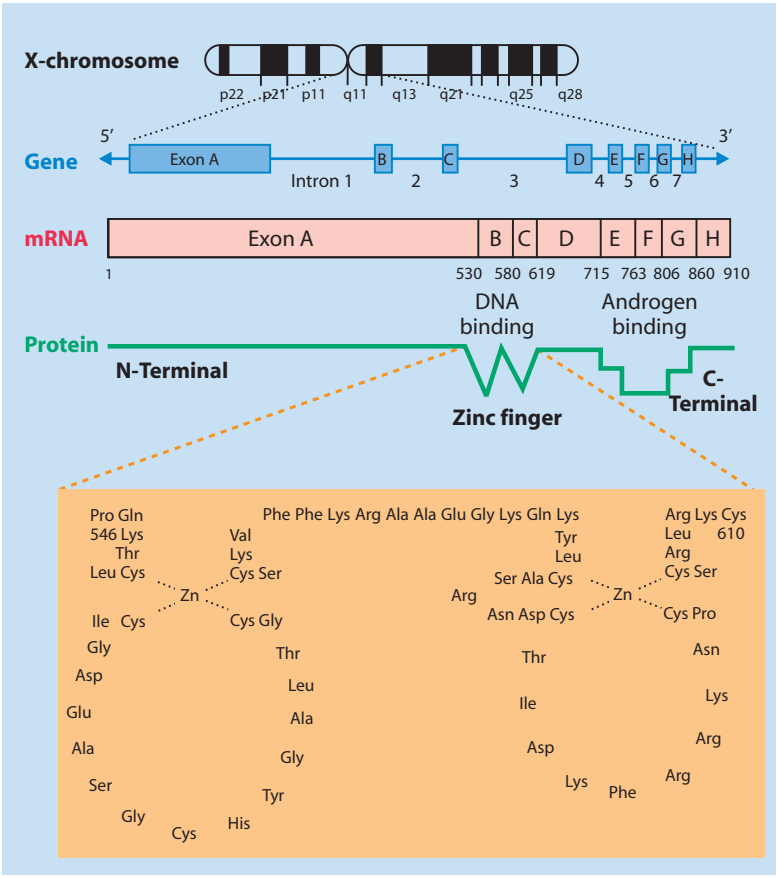

Figure 1.7: Location of the androgen receptor gene on the X chromosome in section Xq11-12. The gene is made up of eight exons (A-H) with seven introns in between. The mRNA encodes 910 amino acids. The DNA-binding domains (exons B and C) contain the zinc finger, which is presented in the magnified section in terms of its amino acid sequence. The zinc finger configuration is characteristic of all steroid hormone receptors. Exons D-H form the androgen-binding domain.

androgen-sensitive genes and thus influence transcription. The DNA-binding domains of the steroid hormone receptors are largely similar (40-90%); the differences determine the gene specificity of the receptor.

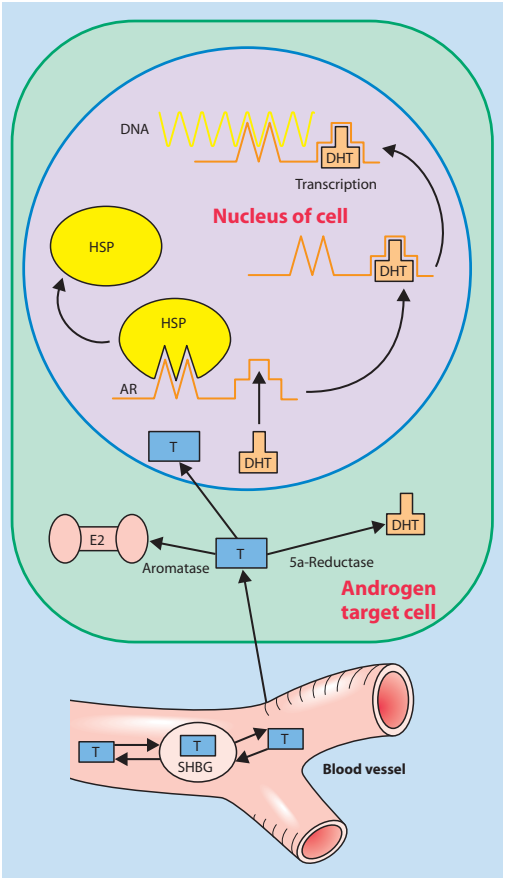

Figure 1.8: Intracellular mechanism of the androgen effect. Testosterone (T) diffuses into the cell and may be metabolized to 5α-dihydrotestosterone (DHT) or estradiol (E2), depending on enzyme availability. T or DHT binds to the androgen receptor (AR) associated with heat shock protein (HSP), which is dimerized after dissociation from HSP (not shown), migrates to the cell nucleus and binds to specific androgen-responsive elements of DNA by means of the zinc finger.

The carboxyterminal end (exons D-H) carries the hydrophobic androgen-binding domain which is responsible for binding testosterone and DHT. This domain has a 40-50% similarity with the amino acid sequence of the corresponding domains of gestagen, mineralocorticoid and corticosteroid receptors. However, there is only slight similarity with the ligand-binding domain of the estrogen receptor. Before binding with an androgen, the androgen receptor is associated with a heat shock protein (HSP 90) (☞ Figure 1.8).

The significance of this association is unknown – HSP 90 may stabilize the androgen receptor or prevent its metabolism. Binding of testosterone or DHT to the androgen receptor induces a conformational change which causes dissociation of the androgen receptor from HSP 90 and activation of the androgen receptor. Activated androgen receptor complexes form dimers and then bind to specific DNA sections known as androgen-responsive elements (AREs), which lie in the promoter region of androgen-sensitive genes. Dimerization increases DNA-binding activity. The first zinc finger, in particular a group of three amino acids at its base, is responsible for specificity of the DNA binding[59]. The second zinc finger stabilizes androgen receptor binding. Binding of the androgen receptor to DNA influences the transcription of androgen-sensitive genes, which regularly lie downstream (3') of the ARE[313,240]. The transcriptional activity of the androgen receptor is modulated by numerous co-regulators[150].

Exon A contains a polymorphic polyglutamine (CAG) repeat sequence which normally has between 8 and 35 repeats. This sequence lies inside a split aminoterminal transactivation domain. The number of CAG repeats affects the transcription intensity of the receptor. The more CAG repeats there are the smaller is the activation of the transcription triggered by the androgen receptor. This is because co-activators and androgens bind more strongly to an androgen receptor with short CAG sequences than to androgen receptors with longer CAG repeats. Therefore, an androgen receptor with few CAG sequences mediates a stronger androgen effect than a receptor with many CAG repeats. A particularly large number of CAG repeats (>38) is associated with androgen resistance, and Kennedy's syndrome, a degenerative bulbospinal motor neuropathy (spinocerebellar ataxia type 7)[448] (☞ Section 2.2.4.1).

As the prostate is an androgen-dependent organ, requiring testosterone and its metabolite DHT for growth and development, and the growth of prostate cancer is at least partially regulated by andro-