Androgens

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Androgens represent the main hormones responsible for maintaining hormonal balance and function in the prostate and testis. As they are involved in prostate and testicular carcinogenesis, more detailed information of their active concentration at the site of action is required. Since the introduction of the term intracrinology as the local formation of active steroid hormones from inactive precursors of the adrenal gland, mainly dehydroepiandrosterone (DHEA) and DHEA-S, it is evident that blood circulating levels of sex steroid hormones need not reflect their actual concentrations in the tissue.

Keywords: androgens ; prostate ; testis ; hormones

1. Introduction

The testis is the major source of androgens as well as estrogens in men, while the prostate is one of the main sex steroid targets.

1.1. Prostate

1.1.1. Intraprostatic Androgens

Intraprostatic steroid determination depends on the way and site of sample removal and its procession before end-point determination. While earlier studies required relatively high amounts of tissue (of the order of tens milligrams), recent more sensitive analytical methods such as various combinations of chromatographic techniques with mass spectrometry (MS) need less than 5 mg of tissue, as obtained by needle biopsy. The specificity of the methods and better separation of steroids enable to reduce of the pre-purification steps. In all instances, the immediate deep freezing of the tissue in liquid nitrogen and its storage at −70 °C is crucial since intraprostatic steroids undergo rapid metabolism. Early reports demonstrated that T in the prostate, after releasing from the androgen-binding protein (ABP), is reduced to its main saturated metabolite, dihydrotestosterone (DHT). DHT, compared to testosterone (T), has a higher affinity for the androgen receptor [1].

1.1.2. Prostate Cancer and Androgen Deprivation Therapy

The importance of sex steroids actions for prostate physiology and pathology is emphasized concerning prostate cancer (PCa), which is one of the most frequent neoplasia in men. Androgens (T and DHT) and their metabolites play important roles in the disease. Through androgen/androgen receptor signaling, they are essential for development and function, as well as the proliferation and survival of epithelial cells within the prostate gland. Androgen deprivation therapy (ADT), either chemical, based on affecting hormone biosynthesis and androgen receptor activation or by surgical castration, has been used for decades to inhibit androgen-dependent PCa cell growth. Unfortunately, many patients will later develop hormone-resistant or castration-resistant cancer. On the other hand, despite the very low serum androgen levels, most castration-resistant cancers remain dependent on the androgen receptor signaling pathway. From this point of view, the intratumoral generation of DHT from adrenal androgen precursors, dehydroepiandrosterone (DHEA) and its sulfate and androstenedione, called intracrinology, is of importance [2].[3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20]

In primates and humans, the active steroid hormones can be formed from inactive precursors of the adrenal gland, mainly DHEA and DHEA-S, where the local formation of active androgens depends on the expression of solute carrier organic anion transporter polypeptides (SLCO, formerly organic anion-transporting peptides (OATP)) and several androgen biosynthetic enzymes—sulfatase, 3β -hydroxysteroid dehydrogenases, 17β -hydroxysteroid dehydrogenases, and 5α -reductases—but also androgen metabolizing enzymes, sulfotransferases, and uridine 5′-diphospho-glucuronosyltransferase. Progression of castration-resistant PCa is accompanied by increased expression of steroid-5α-reductase. This enzyme exists in three different isoforms. Both SRD5A1 and SRD5A2 are expressed in the liver, where the latter is highly expressed in the androgen-sensitive tissue such as the prostate and testes. SRD5A2 is associated with

microsomal membranes and facilitates the conversion of T to DHT, which is essential for the development of the normal male accessory sex organs [21][22]. Currently, there is limited evidence that SRD5A3 makes any contribution to the 5α-reduction of androgen in vivo, but rather, it seems to play a role in N-linked protein glycosylation [23].

As demonstrated by in vitro experiments with fresh prostatic tissue, the concentration of DHT decreases by more than half within 2 h of incubation at 37 °C, indicating the further metabolism of DHT. The saturated 5α-androstanediols, either free or conjugated with glucuronic acid, are the main products [24][25][26]. In addition to 5α -reductases, also one 17β -hydrogenase isotype enzyme, namely Aldo-Keto-Reductase 1C3 (full name type 5 17β-hydroxysteroid dehydrogenase (HSD)/prostaglandin (PG) F2α synthase, abbreviated AKR1C3) is implicated in the production of androgens in castration-resistant PCa [27].

1.1.3. 11-Oxygenated Androgens

In connection with adrenal androgens and their role in castration-resistant PCa, until recently, overlooked 11-oxygenated androgens, namely 11β-hydroxyandrostenedione, 11-ketoandrostenedione, 11β-hydroxytestosterone, and 11β-hydroxydihydrotestosterone should be mentioned here. Studies from various research teams brought evidence that 11β-hydroxyandrostenedione, an abundant DHEA metabolite of exclusively adrenal origin (see [28]), serves as the precursor to these latter potent androgens. Their biosynthesis and clinical significance under various clinical conditions were described and reviewed recently [3][4][5].

Using cancer cell lines LNCaP and VCaP, it was proven that they bind to the androgen receptor with affinities close to that of testosterone and dihydrotestosterone and induced both the expression of representative androgen receptor-regulated genes as well as cellular proliferation in the androgen-dependent prostate [6]. Of interest is that these metabolites are less readily deactivated that classical androgens testosterone and DHT and thus remain active longer [3]. Their determination in the prostate tissue may serve as a promising additional marker for PCa development.

1.1.4. Intraprostatic Androgens—A Summary

The biological activity of sex and other steroids in the prostate is influenced by various factors, such as transport mechanisms regulating steroid uptake [7], steroid receptor content, and, last but not least intraprostatic metabolism [25][26][8][9] and environmental factors [10][11]. Consequently, circulating steroid levels may not fully reflect the situation in the prostate, and the measurement of intraprostatic steroid levels is preferred [12]. DHT, T, and also their adrenal androgen precursors are the first at stake. Alternative pathways of androgen biosynthesis from adrenal precursors, including the backdoor pathway and important intermediate products, should be taken into account as well [13].

1.2. The Testis

1.2.1. Intratesticular Androgens and Their Forms

The testis is the main organ responsible for spermatogenesis and sperm maturation. About 90% of the T circulating in men originates from Leydig cells. Leydig and Sertoli cells are both involved in the formation of testicular tissue. The differentiation of Leydig cells, which is responsible for T formation, is based on the interplay between Sertoli cell ligands and Leydig cell receptors [14]. The importance of such communication between testicular cells is further stressed by the existence of intratesticular androgen-binding protein (ABP), secreted by Sertoli cells, which mediates intratesticular transport of active androgens between Sertoli and Leydig cells. ABP is the product of the same gene of plasmatic sex hormone-binding globulin (SHBG) [15][16].

Steroid hormones occur in several forms. As intratesticular androgens concerns, we distinguish free, albumin-bound, androgen-binding protein (ABP)-bound and, since the processed tissue is vascularized, it is also bound to SHBG from the circulation. In addition, there are polar conjugates, mainly sulfates, and glucuronides. The preparation of the sample plays an important role as the organic extraction solvents must completely disrupt the binding of the steroid to the protein.

Not only free steroid hormones but also albumin-bound forms are available to the target tissues for biological activity. Therefore, by bioavailable steroid hormone is meant the sum of free and albumin-bound steroids. Its determination is more relevant than its total concentration (the sum of conjugated and unconjugated steroid hormones). The non-ABP/SHBG-bound steroids can be analyzed using ammonium sulfate saturated solution to precipitate ABP/SHBG together with the steroids bound to it, followed by its separation using centrifugation. Moreover, androgen conjugates with glucuronic or sulfuric acid occur in testes as well.

1.2.2. Regulation of Testicular Steroidogenesis

Testicular steroidogenesis undergoes sophisticated regulation, including both non-genomic and genomic signaling. Although luteinizing hormone (LH) is the main hormone responsible for T production, there are other factors involved that affect LH-induced signaling [14]. The testes are not only sites of sex steroid biosynthesis but also target organs containing androgen receptors [16][17] and the site of further steroid metabolism [21][18][19]. Thus, from the point of view of endocrine/paracrine regulation, the testis represents a microenvironment characterized by its own hormone metabolism and regulatory mechanisms; in this microenvironment, the actual steroid concentration is of crucial importance and may differ considerably from circulating steroid hormone levels [16][20].

1.3. Conclusive Remarks

Since the first reports into the determination of steroids in testicular and prostatic tissues, methods have advanced considerably with respect to both matrix processing and end-point measurement. However, it is clear from the literature that tissue is sometimes mishandled, particularly prostate tissue, and/or that the most precise measurement method is not always used. This means that a review of the methods used for the analysis of testicular and prostatic steroids is much needed.

2. Determination of Intraprostatic and Intratesticular Androgens

The diagnosis of PCa, BPH, and male fertility disorders involves the analysis of main androgens. Since blood serum does not always reflect the actual situation in affected tissues, as repeatedly evidenced by poor correlation between intratissue and blood steroid levels, the determination in the tissues is to be preferred. For this reason, we have summarized the state of the art in using the intratissue concentrations of the main sex steroid players for diagnostic purposes.

We have shown that the methodical approaches chosen at each stage (material collection, preanalytical processing, final steroid determination) of the analysis of intraprostatic and intratesticular steroids is critical.

The following conclusions may be drawn from the reviewed data and presented tables:

- Advanced analytical tools, such as LC- or GC-MS/MS, best facilitate the analysis of small amounts of prostatic tissue from needle biopsy, as well as providing a more patient-friendly approach.
- DHT is the main intraprostatic androgen, its concentration being about ten times that of blood serum.
- Although intraprostatic DHT is higher in patients with PCa than in other groups in most instances, available data from various researcher groups are not sufficient to distinguish definitely patients with PCa from other subjects. The results strongly depend on methodology, namely analyzed material, sample collection, separation techniques, and end-point measurement. Moreover, there is very little information from healthy men. Only results obtained by the same or similar methodology can be compared seriously.
- T is the main intratesticular androgen, its concentration exceeding blood levels by almost two orders of magnitude, but very little information is available from men with fertility disorders.
- There is no consensus as to whether intratesticular T concentrations correlate with serum levels.
- Intratesticular androgens respond either poorly or not at all to exogenous hormone administration.
- 11-oxygenated androgens as dominant active androgens are promising biomarkers in the evaluation and diagnosis of androgen-dependent diseases. Of crucial importance is their contribution to CRPC progression.

Taken together, these conclusions show that more data are needed on the intraprostatic androgens. Such data are required for both BPH and PCa patients and, in the latter case, they should factor in disease severity. Crucially, such data need to be obtained using a standardized methodology, such as needle biopsy with LC-MS/MS. Furthermore, greater focus on determining the levels of a wider range of intratesticular steroid metabolites, such as those of adrenal origin, would enhance our understanding of other biosynthetic pathways and thereby could contribute to the improved diagnosis of PCa and male fertility disorders.

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