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Transporter for sulfated steroid hormones in the testis − expression pattern, biological significance and implications for fertility in men and rodents

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ABSTRACT

In various tissues, steroid hormones may be sulfated, glucuronidated or otherwise modified. For a long time, these hydrophilic molecules have been considered to be merely inactive metabolites for excretion via bile or urine. Nevertheless, different organs such as the placenta and breast tissue produce large amounts of sulfated steroids. After the discovery of the enzyme steroid sulfatase, which is able to re-activate sulfated steroids, these precursor molecules entered the focus of interest again as a local supply for steroid hormone synthesis with a prolonged half-life compared to their unconjugated counterparts. The first descriptions of this so-called sulfatase pathway in the placenta and breast tissue (with special regards to hormone-dependent breast cancer) were quickly followed by studies of steroid sulfate production and function in the testis. These hydrophilic molecules may not permeate the cell membrane by diffusion in the way that unbound steroids can, but need to be transported through the plasma membrane by transport systems. In the testis, a functional sulfatase pathway requires the expression of specific uptake carrier and efflux transporters in testicular cells, i.e. Sertoli, Leydig and germ cells. Main focus has to be placed on Sertoli cells, as these cells build up the blood-testis barrier.

In this review, an overview of carrier expression pattern in the human as well as rodent testis is provided with special interest towards implications on fertility.

1. Introduction

In contrast to unconjugated steroid hormones, sulfated steroid hormones were considered inactive steroid metabolites without biological significance. Whereas unconjugated steroid hormones can traverse the cellular membrane of target cells by diffusion, bind to cellularly located steroid hormone receptors and promote or inhibit gene transcription in the cell nucleus (for review, see [1]), sulfated steroids may not pass through the cell membrane due to their negative charge at physiological pH and were therefore considered excretion metabolites formed in the liver or kidney [2–6].

Nevertheless, sulfated steroids have been known for about four decades to be synthesized in the reproductive organs in humans and animals. Examples of this are considerable amounts of estrone sulfate (E_1S) in the bovine and equine placenta [7,8], and E_1S secretion from the testes of boars and stallions [9,10]. However, in humans, sulfated steroids are also produced in high amounts in the testis, e.g. pregnenolone sulfate (PREGS), dehydroepiandrosterone sulfate (DHEAS) and testosterone sulfate [11–13]. Apart from the pure excretion rate of these metabolites, many studies have shown that they may be used as precursors for testosterone synthesis in the human testis both *in vivo* and *in vitro* [14–19].

The cleavage enzyme involved in the re-activation of sulfated steroid hormones is steroid sulfatase (STS), also known as arylsulfatase, which is widely distributed in healthy human tissues and in cancer [20]. This has been demonstrated, for example, in human breast cancer: the hydrolysis of sulfated steroids like E_1S and DHEAS is thought to be involved in the local supply of estrogens and androgens in these cancer tissues [21,22]. The re-activation of steroid sulfates, their subsequent binding to classical steroid hormone receptors such as androgen receptor (AR) and estrogen receptors (ERs), and binding to hormone-responsive elements in the DNA leading to gene expression activation or

Abbreviations: ABC, transporter; ATP, binding cassette transporter; AR, androgen receptor; ASBT, apical sodium-dependent bile acid transporter; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; BT, Bblood-testis barrier; CS, cholesterol sulfate; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; E_1S, estrone sulfate; ESRs, estrogen receptors; MDR1, multidrug resistance carrier 1 (gene ABCB1); MRPs, multidrug resistance related proteins (genes ABCCs); NBD, nucleotide binding domain; NTCP, Na^+/taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptides; OSCP1, organic solute carrier protein 1; P-gp, P-glycoprotein (also known as MDR1, gene ABCB1); PREGS, pregnenolone sulfate; SCO, Sertoli-cell only syndrome; SLC, solute carrier family; SNP, single nucleotide polymorphism; SOAT, sodium-dependent organic anion transporter (gene SLC10A6); STS, steroid sulfatase; TMD, transmembrane domain

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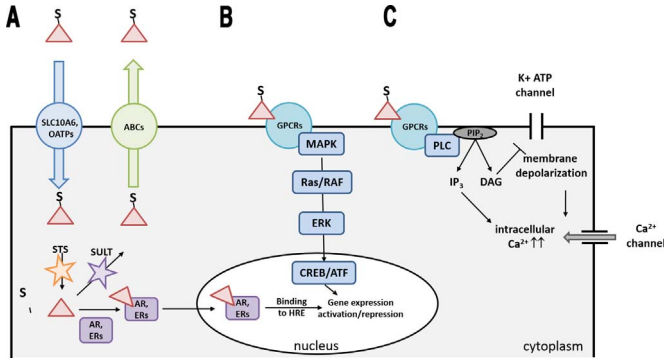

Fig. 1. Schematic drawing of the sulfatase pathway and signaling pathways for sulfated steroid hormones.

A For intracellular action, sulfated steroid hormones (red triangle plus "S") have to be taken up by specific uptake carriers (blue, e.g. SLC10A6, OATPs) into the cell. There, the sulfate residue is cleaved by the activity of STS (orange star). Afterwards, unconjugated steroid hormones (red triangle) are able to bind to nuclear steroid receptors (AR, ERs), translocate into the nucleus and bind to hormone responsive elements (HREs). By this, gene expression may be activated or repressed. This is considered as the genomic pathway as reviewed by Walker [1]. Within the cell, unconjugated steroid hormones can be inactivated by adding a sulfate residue (catalyzed by sulfotransferase (SULT), purple star). As conjugated steroid hormones have longer half-lives, they are considered as a local supply. They may also leave the target cell by specific efflux transporters (green, ATP binding cassette transporters, ABCs).

B Sulfated steroid hormones can also bind to membrane-associated G-protein coupled receptors (GPCRs, e.g. GPER [31], Gnα11 [34,35]) and activate a non-genomic pathway as reviewed by Walker [33]. Activating of MAP kinase (MAPK) cascade via Ras/RAF and ERK leads to phosphorylation of the transcription factors CREB/ATF which act on CREB inducible genes. This pathway has already been shown to be initiated by DHEAS binding [35].

C A second possible non-genomic pathway of steroid hormone action is the depolarization of cell membranes leading to a Ca^{2+} influx. By binding of steroid hormones to membrane receptors (probably GPCRs), phospholipase C (PLC) is activated and cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacyl glycerol (DAG). DAG inhibits K^+ATPase channels, which leads to a Ca^{2+} influx. Furthermore, IP_3 binds to IP_3 receptors on the endoplasmic reticulum which leads to a Ca^{2+} influx. This has already been described for testosterone action in Sertoli cells (for review, see [33]).

repression has been termed the sulfatase pathway (Fig. 1A). As presented later by Selcer et al. [20], STS expression is especially high in hormone dependent breast cancer subtypes. This made human STS a valuable drug target for the treatment of these hormone-dependent cancers [23]. Besides breast tissue, STS is also expressed in other organs, such as the skin or testis. A deficiency of STS leads to X-linked ichthyosis due to an accumulation of cholesterol sulfate (CS) in the skin, and boys with this deficiency were thought to be more likely to suffer from testicular cancer and maldescensus of the testis [24,25]. However, as recently reviewed by Elias et al., the incidence of cryptorchidism does not exceed 5–10%, and the association of testicular cancer and X-linked ichthyosis has not been confirmed [26]. The discovery of transporting systems for organic anions in the testis has increased the evidence of a functional sulfatase pathway in the testis and encouraged further investigations.

In addition to the *de novo* synthesis of steroid hormones in the testis, a second *para*-, intra- and autocrine system for the control of reproductive processes might exist [27,28]. Possible target cells in the testis (Leydig, Sertoli, and germ cells) can be either involved in steroid hormone synthesis (interstitial Leydig cells, for review see [29]) and/or targets of their effects. The latter is due to the expression of classical steroid hormone receptors such as the AR in Leydig and Sertoli cells (for review, see [1,30]) and the two subtypes of ERs (ESR1 and ESR2) in Leydig and Sertoli cells, and distinct germ cell populations [31] (for review, see [32]). A high expression of steroid hormone receptors and STS in the testis contributes to the hypothesis of a functional sulfatase pathway in the normal testis, implying a biological significance of sulfated steroid hormones in normal spermatogenesis [21]. Additionally, sulfated steroids are also able to act directly on target cells by binding to G-protein coupled receptors (GPCRs) in the cell membrane. Activation of these receptors leads to non-genomic signaling pathways, e.g. by activation of the MAP kinase cascade or Ca^{2+} influx (for review, see [33]) (Fig. 1B, C). This has already been demonstrated for DHEAS in rat Sertoli cells [34] and the spermatogenic germ cell line GC-2 [35].

In addition to the enzymatic equipment, testicular cells also have to provide distinct transport systems for a targeted uptake and the release of sulfated steroid hormones as these molecules are not able to pass the lipophilic cell membrane by diffusion. This missing link persistently questioned the existence of a sulfatase pathway in the testis and other organs. The description of the solute carrier family (SLC), located in membranes of different cell types and specifically transporting organic anionic molecules, e.g. bile acids in the liver and gut, has opened up a new discussion about sulfated steroid hormones and their implications for testicular biology. Exclusive to the testis, the transport of sulfated steroids towards the germ cells has to take place across the blood-testis barrier (BTB). The existence of the BTB was first confirmed 1970 by Dym and Fawcett [36] in the rat and 1989 in human testicular biopsies by Bergmann et al. [37]. The BTB is established together with the first wave of spermatogonia developing into spermatocytes which enter meiosis [38] and built in the basal third of the seminiferous epithelium by two adjacent Sertoli cells. Establishment of the BTB is considered a sign of Sertoli cell maturation [39]. It is located above migrating preleptotene spermatocytes and is comprised of tight and gap junctions as well as desmosomes between adjacent Sertoli cells. The BTB subdivides the seminiferous epithelium into two compartments: the basal compartment containing spermatogonia and preleptotene primary spermatocytes, and the adluminal compartment containing successive stages of germ cell development. Junctions between Sertoli cells are tight junctions, basal ectoplasmic specializations, and desmosome-gap junctions (for review, see [40]). The BTB is one of the tightest blood-tissue barriers in the human body, since it protects germ cells in a critical phase of development from white blood cells, as well as harmful toxicants, and regulates the inflow of physiological compounds like water, hormones or nutrients (for review, see [41]).

2. Uptake carrier and efflux transporter for sulfated steroid hormones – structure and function

This review is focused on the transporter superfamilies SLC (Solute Carriers) and ABC (ATP-binding cassette carriers). Members of these families are known to transport sulfated steroid hormones.

The SLC superfamily is comprised of secondary active ion-coupled symporters and anti-porters as well as uniporters, acting by facilitated diffusion [42] located in the plasma membrane and membranous compartments. Currently, the HUGO Nomenclature Committee (HGNC) provides data for 52 SLC families with approximately 395 transporter genes in humans (http://www.genenames.org/cgi-bin/genefamilies/set/752). The first members of the SLC10 family (sodium bile acid co-transporter family) to be described were Na^+/taurocholate co-transporting polypeptide (gene *SLC10A1*, NTCP, located in the liver) and the apical sodium-dependent bile acid transporter (gene *SLC10A2*, ASBT, located in the ileum) [43,44]. Both are essential transporters for the enterohepatic circulation of bile acids. The driving force for both NTCP and ASBT is Na^+ gradients. As reviewed by Hagenbuch and Dawson [43], computer-based analyses suggested a seven-transmembrane topology for all members of the SLC10 family, but a nine-transmembrane structure based on experimental evidence for NTCP and ASBT is more likely [45,46] (for review, see [43]) (Fig. 2A). As shown by Schroeder et al. [47], NTCP also reveals transport capacity for sulfated steroid hormones, namely E_1S. Apart from NTCP and ASBT, some novel members of the SLC10 family have been identified and were classified as *SLC10A3-SLC10A7* [44]. Most still represent orphan carriers, because no substrates have been identified to date, but species-specific