Roles of Steroid Sulfatase in Brain and Other Tissues

a minireview

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Summary

Steroid sulfatase (EC 3.1.6.2) is an important enzyme involved in steroid hormone metabolism. It catalyzes the hydrolysis of steroid sulfates into their unconjugated forms. This action rapidly changes their physiological and biochemical properties, especially in brain and neural tissue. As a result, any imbalance in steroid sulfatase activity may remarkably influence physiological levels of active steroid hormones with serious consequences. Despite that the structure of the enzyme has been completely resolved there is still not enough information about the regulation of its expression and action in various tissues. In the past few years research into the enzyme's properties and regulations has been strongly driven by the discovery of its putative role in the indirect stimulation of the growth of hormone-dependent tumors of the breast and prostate.

Keywords: steroid sulfatase, enzyme, sulfate, physiological role, action

Introduction

Steroid sulfatase (STS) is an almost ubiquitous enzyme of steroid metabolism. It is an important factor influencing many physiological and pathophysiological processes regulated

by steroid hormones. A number of them have been revealed recently (Stanway *et al.* 2007, Hazan *et al.* 2005, Weidler *et al.* 2005, Nakamura *et al.* 2006). Below, the main information on its occurrence, molecular biology and actions are reviewed with emphasis on its physiological and pathophysiological consequences in brain and nervous tissue.

Steroid sulfatase gene and protein

Steroid sulfatase belongs to a superfamily of sixteen different mammalian sulfatases (Obaya 2006). The gene encoding STS is pseudoautosomal in mice, but not in humans, and escapes X inactivation in both species. The functional nucleotide sequence maps to Xp22.3-Xpter (Yen *et al.* 1987, Meroni *et al.* 1996).

The DNA sequence coding for STS on the X-chromosome was cloned, sequenced and well characterized. The 2.4 kbp cDNA encodes a protein of 583 amino acid residues with a short signal peptide of 21-23 amino acids and four potential N-glycosylation motifs N-X-S/T. Probably two of these sites (N_{47} and N_{259}) are used for N-linked oligosaccharide connection.

STS is synthesized as a membrane-bound 63.5 kDa polypeptide. Newly synthesized polypeptide is processed to a mature 61 kDa form. The decrease in size is due to the processing of the oligosaccharide chains, which are cleavable by endoglucosaminidase as well as mannosidase(s) (Conary *et al.* 1986). Recently, STS was purified and crystallized from the membrane bound part of the human placenta (Hernandez-Guzman *et al.* 2001) and shortly after, three-dimensional structures of three human sulfatases were released (Ghosh 2005).

Concerning membrane topology, the enzyme has two membrane-spanning antiparallel hydrophobic α-helices with proline 212 serving as a turn point at the cytosolic side of the endoplasmic reticulum membrane. The polar catalytic domain of the enzyme is situated to the lumen side of the lipid bilayer (Hernandez-Guzman *et al.* 2003). Nevertheless, participation of

the lipid bilayer in maintenance of the active site integrity, passage of the substrate and/or product release was still not exactly determined.

Further details concerning the regulation of STS gene expression on transcriptional and translational levels are beyond the scope of this paper. These issues were excellently reviewed in more comprehensive and specialized articles (Nussbaumer *et al.* 2005, Reed *et al.* 2004, Richard 2004).

Enzyme activity

Sulfatases are a unique group of enzymes that carry at their catalytic site a post-translational modification, an alpha-formylglycine residue that is essential for enzyme activity. Formylglycine is generated by oxidation of a highly conserved cysteine or, in some prokaryotic sulfatases, serine residue (Dierks *et al.* 1998, Miech *et al.* 1998). In eukaryotes, this oxidation occurs in the endoplasmic reticulum during or shortly after import of the nascent sulfatase polypeptide. The mechanisms that are responsible for the oxidation are probably associated with the protein translocation apparatus. *In vitro* studies for arylsulfatase A revealed short linear motif (CTPSR), starting with the cysteine residue, which acts as a target site for the post-translational modification. Proline and arginine play crucial roles for appropriate modification as well as seven additional residues (AALLTGR) directly following the CTPSR sequence (Dierks *et al.* 1999, Knaust *et al.* 1998). The proposed catalytic mechanism also includes the presence of bivalent Ca^{2+} rather than Mg^{2+} cation in the active site of the enzyme. Therefore, the reaction mechanism, which STS uses for steroid sulfate hydrolysis, closely resembles the mechanism that was proposed for arylsulfatases (Boltes *et al.* 2001).

Recent results show that also N-terminal and C-terminal regions have important contribution to the STS enzyme activity (Sugawara *et al.* 2006).

Subcellular localization

In the above mentioned studies STS was found mainly in the rough endoplasmic reticulum. Furthermore, in cultured human fibroblasts, immunohistochemistry proved the enzyme exists in Golgi cisternae and in the trans-Golgi, where it is co-distributed with lysosomal enzymes and the mannose 6-phosphate receptor. STS was also found at the plasma membrane (Kawano *et al.* 1989) and in the coated pits, endosomes and multivesicular endosomes. These structures may be the sites where sulfated estrogen and/or androgen precursors are hydrolyzed. It also co-localizes with lysosomal enzymes and the mannose 6-phosphate receptor here. Despite microsomal STS and lysosomal enzymes sharing several cellular compartments, in the first studies the polypeptide was surprisingly not detected in lysosomes (Willemsen *et al.* 1988). One year later, however, STS activity was also found in lysosomes (Stein *et al.* 1989). On the other hand, sulfatase activity in these compartments and the appropriate protein was isolated and characterized as N-acetylgalactosamine-4-sulfatase (Bond *et al.* 1997). The stability of sulfatases in subcellular compartments with low pH is possibly due to their membrane topology. It could be related to the high homology of two the luminal domains of STS with the lysosomal sulfatases, arylsulfatase A, and arylsulfatase B. However, the transport between trans-Golgi and lysosomes possibly uses a mannose receptor-independent mechanism. This was proposed as a result of the absence of mannose-6-phosphate in STS carbohydrate moiety (Stein *et al.* 1989).

Occurrence in tissues

Enzymatic activity of STS was demonstrated for the first time in rat liver microsomes (Dodgson *et al.* 1954). Later, this enzyme was found in testis (Payne *et al.* 1969), ovary (Clemens *et al.* 2000), adrenal glands, placenta, prostate, skin, kidney, fetal lung (Hobkirk *et*

al. 1982), viscera, endomerium, aorta, bone, peripheral blood leukocytes (Han *et al.* 1987) and brain tissue (Iwamori *et al.* 1976). Now STS is believed to be an almost ubiquitous enzyme. Tissue and organ distribution varies considerably between different mammalian species. Placenta was found to be the richest source of STS. However, the findings differ in dependence on methodological approaches. Immunohistochemistry in combination with reverse transcriptase polymerase chain reaction (RT-PCR) were laregely used for STS localization. Biochemically, sulfatase activity was detected in microsome fraction and/or tissue homogenates. These approaches have been used for identification and characterization of STS activity in rat testis (Payne *et al.* 1969), ovarian granulosa cells (Clemens *et al.* 2000), leukocytes (Han *et al.* 1987) and brain tissue (Iwamori *et al.* 1976). More recently, a biochemical approach was used for determination and characterization of STS activity in *Macaca* brain regions (Kriz *et al.* 2005)

At first there was confusion between with aryl sulfatase C and STS because these enzymes seemed to be different, but recent biochemical and genetic analyses have confirmed that there is only one enzyme (Keinanen *et al.* 1983, Ruoff *et al.* 1991).

Although there was found only one gene for STS, evidence has been found that two different isoforms (microsomal and nuclear) exist in rodents (Nelson *et al.* 1983) and also two isoforms in humans (Chang *et al.* 1990, Munroe *et al.* 1987), probably due to different post-translational modifications.

STS and cholesterol supplementation

It is necessary to mention the involvement of STS in steroidogenic acute regulatory protein (StAR) activity regulation. This 30 kDa phosphoprotein which is derived from its larger precursor plays a crucial role in the intra-mitochondrial transport of cholesterol (Stocco 2001). Cholesterol can be further cleaved to pregnenolone (Pregn), which serves as a

precursor in steroid hormone synthesis and itself acts also as neuroactive steroid. The process occurs in the mitochondrial matrix and is associated with the inner membrane. Experiments on monkey kidney COS-1 cells showed that STS in the presence of StAR and cytochrome P450 (CYP11A1) significantly increased Pregn production in the reaction medium. It is a result of both an increase in StAR translation and prolonged StAR half-life (Sugawara *et al.* 2004). The importance of STS lies in conversion of cholesterol sulfate (CholS) to free cholesterol which is a substrate for CYP11A1. Furthermore, CholS has an inhibitory effect on steroidogenesis in rat adrenal mitochondria by inhibiting the cholesterol transport inside the mitochondrion (Xu *et al.* 1989, Lambeth *et al.* 1987). Therefore, STS prevents CholS inhibitory effect on cholesterol intra-mitochondrial transport. Prolonged StAR half-life might also be explained by STS action on CholS. Released intracellular cholesterol binds StAR and may slightly change its conformation and therefore increases protein half-life. The same effect was observed in MLN 64 transfected cells. This gene contains StAR-homology domain and is expressed in placental mitochondria and lysosomes (Bose *et al.* 2000, Zhang *et al.* 2002).

This phenomenon is probably not only limited to steroidogenic tissues but occurs widely throughout the body. For advances in intracellular cholesterol trafficking and the role of StAR see some of the recent reviews (Strauss *et al.* 1999).

STS and central nervous system

Steroid hormones, regardless of their origin, exert a wide variety of biological effects on the nervous system. They play an important role in the growth, development, maturation and differentiation of the central and peripheral nerves. Furthermore, it was demonstrated that there is an association between STS activity and behavior, as shown for instance in male mice (Le Roy *et al.* 1999). Evidence has been found that brain tissue was able to synthesize various steroid hormones such as Pregn and DHEA from cholesterol *in situ* (Baulieu *et al.* 1990). In

brain tissue, steroids act mainly in autocrine and/or paracrine ways. It was found that cerebral concentrations of Pregn and DHEA neither respond to the administration of adrenocorticotropic hormone nor undergo circadian variations as do circulating adrenal steroids (Corpechot *et al.* 1981). A new term neurosteroids was suggested for these steroids.

Neurosteroids include steroid hormones or their precursors that are newly synthesized from cholesterol or other early precursors in the nervous system. These steroids could be detected in measurable amounts even if peripheral steroidogenic glands were removed (Robel *et al.* 1995).

Neurosteroids occur in the nervous system as free unconjugated steroids, sulfate esters or fatty acids esters (Jo *et al.* 1989). The conjugated forms of steroids frequently exceed those of free steroids and differ in their metabolic, behavioral and also psychological effects such as stress, anxiety, cognition and sleep (Baulieu *et al.* 1996, Majewska 1992). Besides the action of neurosteroids at the transcriptional level, these steroids may affect the nerve cells in a nongenomic way via alteration of neuronal excitability by modulating the activity of several neurotransmitter receptors such as γ-amino butyrate ($GABA_A$) receptors, N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate receptors, nicotinic, muscarinic and σ-receptors (Klangkalya *et al.* 1988, Monnet *et al.* 1995, Valera *et al.* 1992, Wu *et al.* 1991, Shirakawa *et al.* 2005, Wu *et al.* 1997).

Concerning the role of STS, it maintains the balance between sulfated neurosteroids and their unconjugated forms, which in many cases act in opposite ways. The assumption of a steroid binding site at these ligand-gated channels is based on pharmacological studies concerning the strong stereoselectivity and the structure-activity relationship of the action of neuroactive steroids at these neurotransmitter receptors (Lambert *et al.* 1995). In addition, neurosteroids affect these receptors as non-competitive antagonists. It means that steroid-

binding sites are neither situated inside the ion channel nor compete with receptor agonists for their binding sites (Dingledine *et al.* 1999). These various sites, located on the outer receptor surface, may be occupied by various steroids resulting in enhancing or decreasing leakage of a particular ion channel. STS maintains the levels of DHEA/DHEAS and Pregn/pregnenolone sulfate (PregnS), which are the most common neuromodulators. Furthermore, unconjugated and sulfated steroid molecules act in many cases in opposite ways on the same receptor. This fact further emphasizes the role of STS in regulation of the equilibrium between sulfated and free neuroactive steroids in brain.

Glutamate receptors may have also other outputs: soluble tyrosine kinases activation and/or mitogen-activated protein kinase pathways activation. Furthermore, NMDA receptors in plasma membranes could be coupled to NO synthase (NOS) through the postsynaptic density protein 95 scaffold (Tochio *et al.* 2000). Treatment with DHEA causes NMDA stimulation which leads to a decrease in Ca^{2+}-sensitive NOS activity. Therefore, NO production is inhibited and as a consequence DHEA exerts a neuroprotective effect on cultured rat hippocampal neurons (Kurata *et al.* 2004).

Imbalance in the unconjugated steroid/sulfate ratio was shown to influence memory and long-term potentiation in the hippocampus (Flood *et al.* 1992, Yoo *et al.* 1996). Experiments on rats suggest that STS inhibition may become an important tool for enhancing neuronal functions, such as memory, mediated by excitatory neurosteroids. This is caused by increased DHEAS rather than DHEA level, which positively influences brain cholinergic function and leads to memory enhancement (Rhodes *et al.* 1997, Li *et al.* 1997).

PregnS and DHEAS were found to display completely different (antagonistic) properties on $GABA_A$ receptors. Both also exert complex effects at NMDA receptors (Zorumski *et al.* 2000). Therefore, these steroids might possess nootropic properties. Indeed, some studies suggested that intracerebroventricular administration of Pregn led to better

results in various memory tasks in rodents (Flood *et al.* 1992). Furthermore, DHEA was also found to enhance memory retention in mice (Flood *et al.* 1988). These results are likely related to STS levels in used animal models.

In humans it is well known that DHEA serum levels decreases with age (Sulcova *et al.* 1997). This phenomenon was also reported for DHEAS in patients suffering from Alzheimer's disease and multi-infarct dementia (Sunderland *et al.* 1989, Yanase *et al.* 1996). Unfortunately, direct information on DHEA/DHEAS ratio and STS levels is still missing. To date no study is available concerning STS activity and its relationship to dementia disorders. Studies on the determination of neurosteroids in cerebrospinal fluid may prove promising (Kim *et al.* 2003).

PregnS and DHEAS were also suggested to be involved in the development of tolerance to ethanol in mice (Barbosa *et al.* 2001). Nevertheless, a connection between ethanol tolerance and STS activity was not demonstrated.

DHEA was also found to have important effects in differentiation of glia and neurons during their development (Roberts *et al.* 1987) and in neocortical organization during development suggesting that DHEA may have trophic factor-like activity (Compagnone *et al.* 1998). Neuronal remodeling can be also explained by DHEA interaction with various neurotransmitter systems (Lapchak *et al.* 2001).

Furthermore, it was proposed that neurosteroids could act on nerve cells via hypothesized surface receptors coupled with G-proteins or through specific membrane sites using calcium as an intracellular messenger (Orchinik *et al.* 1992, Ramirez *et al.* 1996). Recently, it was showed that σ 1 receptor-like protein activity was modulated by PregnS. This protein is coupled with $G_{i/o}$ protein, and as a result this interaction leads to enhancement in short-term presynaptic facilitation onto adult hippocampal CA1 neurons (Schiess *et al.* 2005). As was previously mentioned, PregnS levels are among others under the control of STS.

The detailed STS localization in brain tissue has not been exactly determined. Most studies are limited only to major brain regions (Steckelbroeck *et al.* 2004, Kriz *et al.* 2005). Subcellular localization of STS activity in brain tissues has not been clearly identified. However, it is generally supposed that subcellular STS activity distribution does not differ significantly from the distribution in other cells.

Preliminary results from our laboratory concerning STS activity in several types of human brain tumors showed surprising differences in STS activity in relation to tumor type. Neither age nor sex of studied subjects significantly influenced obtained results.

The roles of STS in physiology and pathophysiology

STS and breast cancer

Much information concerning the role of STS in breast cancer and its treatment have been published (Sasano *et al.* 2006, Stanway *et al.* 2007, Tsunoda *et al.* 2006). Besides 17β-hydroxysteroid dehydrogenase (17β-HSD) type I and aromatase, STS belongs to the most important targets for potential endocrine therapy in humans (Suzuki *et al.* 2005). As such, STS has no direct effect on tumor progression and development. However, it maintains the equilibrium between sulfated and unconjugated steroids whose effects in breast cancer development are, in many cases, completely different (Reed *et al.* 2004).

In hormone-dependent human breast cancer, 17β-estradiol (E_2) contributes highly to tumor growth and development. Furthermore, some carcinoma cells actually require estrogens (especially E_2) for their growth (Sasano *et al.* 2006). The importance of STS in intratumoral estrogen production lies in its crucial role in the conversion of estrone sulfate (E_1S) to estrone (E_1) (Pasqualini 2004). E_1 could be further metabolized to E_2 via the action of 17β-HSD type I (Poutanen *et al.* 1995). Sulfated estrogens are unable to bind to estrogen receptors and thus

they are inactive as hormones (Pasqualini *et al.* 1989). On the other hand, sulfation rapidly changes the polarity of the hydrophobic steroid ring and therefore is widely used for hormone transport. Furthermore, sulfated steroid conjugates act as a reservoir of active hormones and/or their precursors via STS action (Reed *et al.* 2004).

In postmenopausal women, a large contribution to estrogen level comes from the aromatization of circulating androgens (Reed *et al.* 1979). In androgen biosynthesis, STS also plays important role in conversion of DHEAS to DHEA, while DHEA serves as precursor for androstenedione and its further aromatization as shown in Fig. 1 (Nakata *et al.* 2003, Suzuki *et al.* 2003). Therefore, STS may affect E_2 biosynthesis on two levels: conversion of DHEAS to DHEA and E_1S to E_1.

The importance of STS in intratumoral estrogen production has prompted the development of new potent STS inhibitors.

STS and dermatopathies

One of the best known diseases that have been ascribed to STS deficiency is X-linked ichthyosis. It is a relatively common genetic disorder, clinically characterized by a generalized desquamation of large, adherent, dark brown scales of the skin. The majority of all cases are caused by a compete deletion of the STS gene from the distal short arm of the X-chromosome (Richard 2004).

Other STS defects are usually caused by one or more point mutations from the seven possible (Ghosh 2004). These mutations lead to disruptions in the active site and/or interference with enzyme's membrane-associating motifs that are crucial for the integrity of the catalytic site. All of these mutations are located near the C-terminal region of the STS enzyme (Ghosh 2004, Alperin *et al.* 1997).

On the other hand, locally enhanced STS activity leads to increased DHEA production, which serves as a precursor of active androgens such as 5α-dihydrotestosterone (DHT) and/or testosterone (Hoffmann 2001). In women, local excess of DHT may cause an androgenic alopecia. In other target tissues (as pubic region and axillary follicles) it may result in hirsutism (Price 2003). Hence, in women with androgenic alopecia STS inhibition can be effective in treating both disorders.

STS action and the immune system

Concerning STS activity and the immune system, maintaining the DHEA/DHEAS ratio further emphasizes the role of STS. Studies on aged mice revealed that *in vitro* DHEA, but not DHEAS, suppressed the release of Th 2 cytokines and therefore enhanced Th 1 response (Daynes *et al.* 1993). Thus, STS present in macrophages (Hennebold *et al.* 1994) may govern the local availability of DHEA and influence the immune response. It is due to the presence of macrophages in lymphoid tissue where Th cell maturation occurs.

Furthermore, the balance of glucocorticoids to DHEA determines the further progression of Th cells to either Th 1 or Th 2 phenotypic line. Cortisol was found to favor the development to Th 2 cells, while DHEA promotes a Th 1 mediated response (Purohit *et al.* 2002).

The enzyme was also found in peripheral blood leukocytes (Han *et al.* 1987), bone (Purohit *et al.* 1992) and thrombocytes (Soliman *et al.* 1993).

Concerning pathological states, many breast and other tumors are infiltrated by lymphocytes and macrophages (Kelly *et al.* 1988). Consequently, it is possible that STS activity of these cells can finally make an important contribution to estrogen synthesis in breast tumors.

Since the STS activity is present in such available tissue, these cells were suggested to provide a relatively simple model for monitoring the efficacy of new STS inhibitors (Purohit *et al.* 1997, Reed *et al.* 1990).

STS action in bone

It was suggested that local formation of estrone could be an important source of estrogens for regulating bone formation (Janssen *et al.* 1999). Further investigations showed that HOS and MG 63 osteoblast cell lines expressed mRNA for STS and were able to convert both DHEAS as well as E_1S (Fujikawa *et al.* 1997). This pathway involving STS was found to be active not only in cells derived from osteosarcoma but also in osteoblast-like cells cultured from bone fragments. In the latter STS activity was two times higher in comparison with osteosarcoma-derived cells (Muir *et al.* 2004).

The existence of steroid sulfatase in human and rat osteoblast cells suggests that osteoblast cells also possess the capacity to convert circulating sulfo-conjugated steroids to more active androgens and especially estrogens (Janssen *et al.* 1999). This may indicate an important contribution of bone in facilitating the hormonal action.

<u>STS activation and inhibition</u>

Recently explored roles of STS in many pathophysiological processes, especially in breast cancer, prompted efforts for preparation of efficient STS modulators, particularly inhibitors. STS inhibitors may be divided in three basic groups:

A. reversible inhibitors

These compounds follow the same strategy: replacement of 3β sulfate moiety in E_1S with another one that mimics sulfate group. Attempts to synthesize these derivatives finally led to

the preparation of a leading compound. Molecules of such inhibitors compete for STS active site with natural substrates.

B. irreversible inhibitors

This group covers the majority of recently reported STS inhibitors and could be further divided to steroidal and non-steroidal irreversible inhibitors. The former group contains estrone-3-O-sulfamate and its derivatives. Despite their efficiency, their use in clinical practice is quite limited because of various side effects. Recently, attention has been focused on non-steroidal derivatives of coumarin and the exploration of a new leading compound 4-methylcoumarin-7-O-sulfamate (COUMATE) (Purohit *et al.* 1996). Further synthetic efforts led to the preparation of efficient inhibitors derived from COUMATE (Woo *et al.* 2000). Some of these were found suitable for clinical practice and entered clinical trials.

C. dual inhibitors

The third direction of STS inhibitor development is focused on compounds with a dual mode of action. These can inhibit both STS and aromatase activities or act as STS inhibitors and antiproliferative or antiangiogenic agents at the same time. According to the mentioned effects they may be especially useful in treatment of hormone-dependent cancers.

A more detailed overview concerning STS inhibitors is beyond the scope of this review. For detailed discussion of the complex molecular structures and mechanisms that govern STS inhibition see other specialized articles (Purohit *et al.* 2003, Nussbaumer *et al.* 2005, Reed *et al.* 2004, Nussbaumer *et al.* 2004).

As demonstrated on the previous pages, steroid sulfatase possesses a broad spectrum of actions, often unexpected. This may influence many physiological processes connected with steroid hormones and their genomic as well as non-genomic effects. Development of

potent agents, inhibitors or activators of this enzyme, represents one of the tools for effective treatment of various disorders caused by (steroid) hormone deregulations.

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REFERENCES

ALPERIN ES, SHAPIRO LJ: Characterization of point mutations in patients with X-linked ichthyosis. Effects on the structure and function of the steroid sulfatase protein. *J Biol Chem* **272**: 20756-20763, 1997.

BARBOSA AD, MORATO GS: Influence of neurosteroids on the development of rapid tolerance to ethanol in mice. *Eur J Pharmacol* **431**: 179-188, 2001.

BAULIEU EE, ROBEL P: Neurosteroids: a new brain function? *J Steroid Biochem Mol Biol* **37**: 395-403, 1990.

BAULIEU EE, ROBEL P: Dehydroepiandrosterone and dehydroepiandrosterone sulfate as neuroactive neurosteroids. *J Endocrinol* **150 Suppl**: S221-239, 1996.

BOLTES I, CZAPINSKA H, KAHNERT A, VON BULOW R, DIERKS T, SCHMIDT B, VON FIGURA K, KERTESZ MA, USON I: 1.3 A structure of arylsulfatase from Pseudomonas aeruginosa establishes the catalytic mechanism of sulfate ester cleavage in the sulfatase family. *Structure* **9**: 483-491, 2001.

BOND CS, CLEMENTS PR, ASHBY SJ, COLLYER CA, HARROP SJ, HOPWOOD JJ, GUSS JM: Structure of a human lysosomal sulfatase. *Structure* **5**: 277-289, 1997.

BOSE HS, BALDWIN MA, MILLER WL: Evidence that StAR and MLN64 act on the outer mitochondrial membrane as molten globules. *Endocr Res* **26**: 629-637, 2000.

CHANG PL, MUELLER OT, LAFRENIE RM, VAREY PA, ROSA NE, DAVIDSON RG, HENRY WM, SHOWS TB: The human arylsulfatase-C isoenzymes: two distinct genes that escape from X inactivation. *Am J Hum Genet* **46**: 729-737, 1990.

CLEMENS JW, KABLER HL, SARAP JL, BEYER AR, LI PK, SELCER KW: Steroid sulfatase activity in the rat ovary, cultured granulosa cells, and a granulosa cell line. *J Steroid Biochem Mol Biol* **75**: 245-252, 2000.

COMPAGNONE NA, MELLON SH: Dehydroepiandrosterone: a potential signalling molecule for neocortical organization during development. *Proc Natl Acad Sci U S A* **95**: 4678-4683, 1998.

CONARY J, NAUERTH A, BURNS G, HASILIK A, VON FIGURA K: Steroid sulfatase. Biosynthesis and processing in normal and mutant fibroblasts. *Eur J Biochem* **158**: 71-76, 1986.

CORPECHOT C, ROBEL P, AXELSON M, SJOVALL J, BAULIEU EE: Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proc Natl Acad Sci U S A* **78**: 4704-4707, 1981.

DAYNES RA, ARANEO BA, ERSHLER WB, MALONEY C, LI GZ, RYU SY: Altered regulation of IL-6 production with normal aging. Possible linkage to the age-associated decline in dehydroepiandrosterone and its sulfated derivative. *J Immunol* **150**: 5219-5230, 1993.

DIERKS T, MIECH C, HUMMERJOHANN J, SCHMIDT B, KERTESZ MA, VON FIGURA K: Posttranslational formation of formylglycine in prokaryotic sulfatases by modification of either cysteine or serine. *J Biol Chem* **273**: 25560-25564, 1998.

DIERKS T, LECCA MR, SCHLOTTERHOSE P, SCHMIDT B, VON FIGURA K: Sequence determinants directing conversion of cysteine to formylglycine in eukaryotic sulfatases. *Embo J* **18**: 2084-2091, 1999.

DINGLEDINE R, BORGES K, BOWIE D, TRAYNELIS SF: The glutamate receptor ion channels. *Pharmacol Rev* **51**: 7-61, 1999.

DODGSON KS, SPENCER B, THOMAS J: Studies on sulphatases. 6. The localization of arylsulphatase in the rat-liver cell. *Biochem J* **56**: 177-181, 1954.

FLOOD JF, SMITH GE, ROBERTS E: Dehydroepiandrosterone and its sulfate enhance memory retention in mice. *Brain Res* **447**: 269-278, 1988.

FLOOD JF, MORLEY JE, ROBERTS E: Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc Natl Acad Sci U S A* **89**: 1567-1571, 1992.
FUJIKAWA H, OKURA F, KUWANO Y, SEKIZAWA A, CHIBA H, SHIMODAIRA K, SAITO H, YANAIHARA T: Steroid sulfatase activity in osteoblast cells. *Biochem Biophys Res Commun* **231**: 42-47, 1997.
GHOSH D: Mutations in X-linked ichthyosis disrupt the active site structure of estrone/DHEA sulfatase. *Biochim Biophys Acta* **1739**: 1-4, 2004.
GHOSH D: Three-dimensional structures of sulfatases. *Methods Enzymol* **400**: 273-293, 2005.
HAN HD, FENCL MM, TULCHINSKY D: Variations in estrone sulfatase activity in human leukocytes. *J Clin Endocrinol Metab* **65**: 1026-1030, 1987.
HAZAN C, ORLOW SJ, SCHAFFER JV: X-linked recessive ichthyosis. *Dermatol Online J* **11**: 12, 2005.
HENNEBOLD JD, DAYNES RA: Regulation of macrophage dehydroepiandrosterone sulfate metabolism by inflammatory cytokines. *Endocrinology* **135**: 67-75, 1994.
HERNANDEZ-GUZMAN FG, HIGASHIYAMA T, OSAWA Y, GHOSH D: Purification, characterization and crystallization of human placental estrone/dehydroepiandrosterone sulfatase, a membrane-bound enzyme of the endoplasmic reticulum. *J Steroid Biochem Mol Biol* **78**: 441-450, 2001.
HERNANDEZ-GUZMAN FG, HIGASHIYAMA T, PANGBORN W, OSAWA Y, GHOSH D: Structure of human estrone sulfatase suggests functional roles of membrane association. *J Biol Chem* **278**: 22989-22997, 2003.
HOBKIRK R, CARDY C, NILSEN M, SAIDI F: Estrone sulfatase activity in guinea pig tissues. *J Steroid Biochem* **17**: 71-76, 1982.
HOFFMANN R: Enzymology of the hair follicle. *Eur J Dermatol* **11**: 296-300, 2001.
IWAMORI M, MOSER HW, KISHIMOTO Y: Steroid sulfatase in brain: comparison of sulfohydrolase activities for various steroid sulfates in normal and pathological brains, including the various forms of metachromatic leukodystrophy. *J Neurochem* **27**: 1389-1395, 1976.
JANSSEN JM, BLAND R, HEWISON M, COUGHTRIE MW, SHARP S, ARTS J, POLS HA, VAN LEEUWEN JP: Estradiol formation by human osteoblasts via multiple pathways: relation with osteoblast function. *J Cell Biochem* **75**: 528-537, 1999.
JO DH, ABDALLAH MA, YOUNG J, BAULIEU EE, ROBEL P: Pregnenolone, dehydroepiandrosterone, and their sulfate and fatty acid esters in the rat brain. *Steroids* **54**: 287-297, 1989.
KAWANO J, KOTANI T, UMEKI K, OINUMA T, OHTAKI S, AIKAWA E: A monoclonal antibody to rat liver arylsulfatase C and its application in immunohistochemistry. *J Histochem Cytochem* **37**: 683-690, 1989.
KEINANEN BM, NELSON K, DANIEL WL, ROQUE JM: Genetic analysis of murine arylsulfatase C and steroid sulfatase. *Genetics* **105**: 191-206, 1983.
KELLY PM, DAVISON RS, BLISS E, MCGEE JO: Macrophages in human breast disease: a quantitative immunohistochemical study. *Br J Cancer* **57**: 174-177, 1988.
KIM SB, HILL M, KWAK YT, HAMPL R, JO DH, MORFIN R: Neurosteroids: Cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics. *J Clin Endocrinol Metab* **88**: 5199-5206, 2003.
KLANGKALYA B, CHAN A: Structure-activity relationships of steroid hormones on muscarinic receptor binding. *J Steroid Biochem* **29**: 111-118, 1988.

KNAUST A, SCHMIDT B, DIERKS T, VON BULOW R, VON FIGURA K: Residues critical for formylglycine formation and/or catalytic activity of arylsulfatase A. *Biochemistry* **37**: 13941-13946, 1998.
KRIZ L, BICIKOVA M, HILL M, HAMPL R: Steroid sulfatase and sulfuryl transferase activity in monkey brain tissue. *Steroids* **70**: 960-969, 2005.
KURATA K, TAKEBAYASHI M, MORINOBU S, YAMAWAKI S: beta-estradiol, dehydroepiandrosterone, and dehydroepiandrosterone sulfate protect against N-methyl-D-aspartate-induced neurotoxicity in rat hippocampal neurons by different mechanisms. *J Pharmacol Exp Ther* **311**: 237-245, 2004.
LAMBERT JJ, BELELLI D, HILL-VENNING C, PETERS JA: Neurosteroids and GABAA receptor function. *Trends Pharmacol Sci* **16**: 295-303, 1995.
LAMBETH JD, XU XX, GLOVER M: Cholesterol sulfate inhibits adrenal mitochondrial cholesterol side chain cleavage at a site distinct from cytochrome P-450scc. Evidence for an intramitochondrial cholesterol translocator. *J Biol Chem* **262**: 9181-9188, 1987.
LAPCHAK PA, ARAUJO DM: Preclinical development of neurosteroids as neuroprotective agents for the treatment of neurodegenerative diseases. *Int Rev Neurobiol* **46**: 379-397, 2001.
LE ROY I, MORTAUD S, TORDJMAN S, DONSEZ-DARCEL E, CARLIER M, DEGRELLE H, ROUBERTOUX PL: Genetic correlation between steroid sulfatase concentration and initiation of attack behavior in mice. *Behav Genet* **29**: 131-136, 1999.
LI PK, RHODES ME, BURKE AM, JOHNSON DA: Memory enhancement mediated by the steroid sulfatase inhibitor (p-O-sulfamoyl)-N-tetradecanoyl tyramine. *Life Sci* **60**: PL45-51, 1997.
MAJEWSKA MD: Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Prog Neurobiol* **38**: 379-395, 1992.
MERONI G, FRANCO B, ARCHIDIACONO N, MESSALI S, ANDOLFI G, ROCCHI M, BALLABIO A: Characterization of a cluster of sulfatase genes on Xp22.3 suggests gene duplications in an ancestral pseudoautosomal region. *Hum Mol Genet* **5**: 423-431, 1996.
MIECH C, DIERKS T, SELMER T, VON FIGURA K, SCHMIDT B: Arylsulfatase from Klebsiella pneumoniae carries a formylglycine generated from a serine. *J Biol Chem* **273**: 4835-4837, 1998.
MONNET FP, MAHE V, ROBEL P, BAULIEU EE: Neurosteroids, via sigma receptors, modulate the [3H]norepinephrine release evoked by N-methyl-D-aspartate in the rat hippocampus. *Proc Natl Acad Sci U S A* **92**: 3774-3778, 1995.
MUIR M, ROMALO G, WOLF L, ELGER W, SCHWEIKERT HU: Estrone sulfate is a major source of local estrogen formation in human bone. *J Clin Endocrinol Metab* **89**: 4685-4692, 2004.
MUNROE DG, CHANG PL: Tissue-specific expression of human arylsulfatase-C isozymes and steroid sulfatase. *Am J Hum Genet* **40**: 102-114, 1987.
NAKAMURA Y, SUZUKI T, FUKUDA T, ITO A, ENDO M, MORIYA T, ARAI Y, SASANO H: Steroid sulfatase and estrogen sulfotransferase in human prostate cancer. *Prostate* **66**: 1005-1012, 2006.
NAKATA T, TAKASHIMA S, SHIOTSU Y, MURAKATA C, ISHIDA H, AKINAGA S, LI PK, SASANO H, SUZUKI T, SAEKI T: Role of steroid sulfatase in local formation of estrogen in post-menopausal breast cancer patients. *J Steroid Biochem Mol Biol* **86**: 455-460, 2003.
NELSON K, KEINANEN BM, DANIEL WL: Murine arylsulfatase C: evidence for two isozymes. *Experientia* **39**: 740-742, 1983.
NUSSBAUMER P, BILLICH A: Steroid sulfatase inhibitors. *Med Res Rev* **24**: 529-576, 2004.

NUSSBAUMER P, BILLICH A: Steroid sulfatase inhibitors: their potential in the therapy of breast cancer. *Curr Med Chem Anticancer Agents* **5**: 507-528, 2005.
OBAYA AJ: Molecular cloning and initial characterization of three novel human sulfatases. *Gene* **372**: 110-117, 2006.
ORCHINIK M, MURRAY TF, FRANKLIN PH, MOORE FL: Guanyl nucleotides modulate binding to steroid receptors in neuronal membranes. *Proc Natl Acad Sci U S A* **89**: 3830-3834, 1992.
PASQUALINI JR, GELLY C, NGUYEN BL, VELLA C: Importance of estrogen sulfates in breast cancer. *J Steroid Biochem* **34**: 155-163, 1989.
PASQUALINI JR: The selective estrogen enzyme modulators in breast cancer: a review. *Biochim Biophys Acta* **1654**: 123-143, 2004.
PAYNE AH, MASON M, JAFFE RB: Testicular steroid sulfatase. Substrate specificity and inhibition. *Steroids* **14**: 685-704, 1969.
POUTANEN M, ISOMAA V, PELTOKETO H, VIHKO R: Role of 17 beta-hydroxysteroid dehydrogenase type 1 in endocrine and intracrine estradiol biosynthesis. *J Steroid Biochem Mol Biol* **55**: 525-532, 1995.
PRICE VH: Androgenetic alopecia in women. *J Investig Dermatol Symp Proc* **8**: 24-27, 2003.
PUROHIT A, FLANAGAN AM, REED MJ: Estrogen synthesis by osteoblast cell lines. *Endocrinology* **131**: 2027-2029, 1992.
PUROHIT A, WOO LW, SINGH A, WINTERBORN CJ, POTTER BV, REED MJ: In vivo activity of 4-methylcoumarin-7-O-sulfamate, a nonsteroidal, nonestrogenic steroid sulfatase inhibitor. *Cancer Res* **56**: 4950-4955, 1996.
PUROHIT A, FROOME VA, WANG DY, POTTER BV, REED MJ: Measurement of oestrone sulphatase activity in white blood cells to monitor in vivo inhibition of steroid sulphatase activity by oestrone-3-O-sulphamate. *J Steroid Biochem Mol Biol* **62**: 45-51, 1997.
PUROHIT A, REED MJ: Regulation of estrogen synthesis in postmenopausal women. *Steroids* **67**: 979-983, 2002.
PUROHIT A, WOO LW, CHANDER SK, NEWMAN SP, IRESON C, HO Y, GRASSO A, LEESE MP, POTTER BV, REED MJ: Steroid sulphatase inhibitors for breast cancer therapy. *J Steroid Biochem Mol Biol* **86**: 423-432, 2003.
RAMIREZ VD, ZHENG J: Membrane sex-steroid receptors in the brain. *Front Neuroendocrinol* **17**: 402-439, 1996.
REED MJ, HUTTON JD, BAXENDALE PM, JAMES VH, JACOBS HS, FISHER RP: The conversion of androstenedione to oestrone and production of oestrone in women with endometrial cancer. *J Steroid Biochem* **11**: 905-911, 1979.
REED MJ, LAI LC, OWEN AM, SINGH A, COLDHAM NG, PUROHIT A, GHILCHIK MW, SHAIKH NA, JAMES VH: Effect of treatment with 4-hydroxyandrostenedione on the peripheral conversion of androstenedione to estrone and in vitro tumor aromatase activity in postmenopausal women with breast cancer. *Cancer Res* **50**: 193-196, 1990.
REED MJ, PUROHIT A, WOO LW, NEWMAN SP, POTTER BV: Steroid Sulfatase: Molecular Biology, Regulation and Inhibition. *Endocr Rev* **26(2)**: 171-202, 2004.
RHODES ME, LI PK, BURKE AM, JOHNSON DA: Enhanced plasma DHEAS, brain acetylcholine and memory mediated by steroid sulfatase inhibition. *Brain Res* **773**: 28-32, 1997.
RICHARD G: Molecular genetics of the ichthyoses. *Am J Med Genet C Semin Med Genet* **131C**: 32-44, 2004.
ROBEL P, BAULIEU EE: Neurosteroids: biosynthesis and function. *Crit Rev Neurobiol* **9**: 383-394, 1995.
ROBERTS E, BOLOGA L, FLOOD JF, SMITH GE: Effects of dehydroepiandrosterone and its sulfate on brain tissue in culture and on memory in mice. *Brain Res* **406**: 357-362, 1987.

RUOFF BM, DANIEL WL: Comparative biochemistry of mammalian arylsulfatase C and steroid sulfatase. *Comp Biochem Physiol B* **98**: 313-322, 1991.
SASANO H, SUZUKI T, NAKATA T, MORIYA T: New development in intracrinology of breast carcinoma. *Breast Cancer* **13**: 129-136, 2006.
SCHIESS AR, PARTRIDGE LD: Pregnenolone sulfate acts through a G-protein-coupled sigma1-like receptor to enhance short term facilitation in adult hippocampal neurons. *Eur J Pharmacol* **518**: 22-29, 2005.
SHIRAKAWA H, KATSUKI H, KUME T, KANEKO S, AKAIKE A: Pregnenolone sulphate attenuates AMPA cytotoxicity on rat cortical neurons. *Eur J Neurosci* **21**: 2329-2335, 2005.
SOLIMAN HR, DIRE D, BOUDOU P, JULIEN R, LAUNAY JM, BRERAULT JL, VILLETTE JM, FIET J: Characterization of estrone sulfatase activity in human thrombocytes. *J Steroid Biochem Mol Biol* **46**: 215-226, 1993.
STANWAY SJ, DELAVAULT P, PUROHIT A, WOO LW, THURIEAU C, POTTER BV, REED MJ: Steroid sulfatase: a new target for the endocrine therapy of breast cancer. *Oncologist* **12**: 370-374, 2007.
STECKELBROECK S, NASSEN A, UGELE B, LUDWIG M, WATZKA M, REISSINGER A, CLUSMANN H, LUTJOHANN D, SIEKMANN L, KLINGMULLER D, HANS VH: Steroid sulfatase (STS) expression in the human temporal lobe: enzyme activity, mRNA expression and immunohistochemistry study. *J Neurochem* **89**: 403-417, 2004.
STEIN C, HILLE A, SEIDEL J, RIJNBOUT S, WAHEED A, SCHMIDT B, GEUZE H, VON FIGURA K: Cloning and expression of human steroid-sulfatase. Membrane topology, glycosylation, and subcellular distribution in BHK-21 cells. *J Biol Chem* **264**: 13865-13872, 1989.
STOCCO DM: StAR protein and the regulation of steroid hormone biosynthesis. *Annu Rev Physiol* **63**: 193-213, 2001.
STRAUSS JF, 3RD, KALLEN CB, CHRISTENSON LK, WATARI H, DEVOTO L, ARAKANE F, KIRIAKIDOU M, SUGAWARA T: The steroidogenic acute regulatory protein (StAR): a window into the complexities of intracellular cholesterol trafficking. *Recent Prog Horm Res* **54**: 369-394; discussion 394-365, 1999.
SUGAWARA T, FUJIMOTO S: The potential function of steroid sulphatase activity in steroid production and steroidogenic acute regulatory protein expression. *Biochem J* **380**: 153-160, 2004.
SUGAWARA T, NOMURA E, HOSHI N: Both N-terminal and C-terminal regions of steroid sulfatase are important for enzyme activity. *J Endocrinol* **188**: 365-374, 2006.
SULCOVA J, HILL M, HAMPL R, STARKA L: Age and sex related differences in serum levels of unconjugated dehydroepiandrosterone and its sulphate in normal subjects. *J Endocrinol* **154**: 57-62, 1997.
SUNDERLAND T, MERRIL CR, HARRINGTON MG, LAWLOR BA, MOLCHAN SE, MARTINEZ R, MURPHY DL: Reduced plasma dehydroepiandrosterone concentrations in Alzheimer's disease. *Lancet* **2**: 570, 1989.
SUZUKI T, MIKI Y, NAKATA T, SHIOTSU Y, AKINAGA S, INOUE K, ISHIDA T, KIMURA M, MORIYA T, SASANO H: Steroid sulfatase and estrogen sulfotransferase in normal human tissue and breast carcinoma. *J Steroid Biochem Mol Biol* **86**: 449-454, 2003.
SUZUKI T, MIKI Y, NAKAMURA Y, MORIYA T, ITO K, OHUCHI N, SASANO H: Sex steroid-producing enzymes in human breast cancer. *Endocr Relat Cancer* **12**: 701-720, 2005.
TOCHIO H, MOK YK, ZHANG Q, KAN HM, BREDT DS, ZHANG M: Formation of nNOS/PSD-95 PDZ dimer requires a preformed beta-finger structure from the nNOS PDZ domain. *J Mol Biol* **303**: 359-370, 2000.

TSUNODA Y, SHIMIZU Y, TSUNODA A, TAKIMOTO M, SAKAMOTO MA, KUSANO M: Steroid sulfatase in breast carcinoma and change of serum estrogens levels after operation. *Acta Oncol* **45**: 584-589, 2006.
VALERA S, BALLIVET M, BERTRAND D: Progesterone modulates a neuronal nicotinic acetylcholine receptor. *Proc Natl Acad Sci U S A* **89**: 9949-9953, 1992.
WEIDLER C, STRUHAROVA S, SCHMIDT M, UGELE B, SCHOLMERICH J, STRAUB RH: Tumor necrosis factor inhibits conversion of dehydroepiandrosterone sulfate (DHEAS) to DHEA in rheumatoid arthritis synovial cells: a prerequisite for local androgen deficiency. *Arthritis Rheum* **52**: 1721-1729, 2005.
WILLEMSEN R, KROOS M, HOOGEVEEN AT, VAN DONGEN JM, PARENTI G, VAN DER LOOS CM, REUSER AJ: Ultrastructural localization of steroid sulphatase in cultured human fibroblasts by immunocytochemistry: a comparative study with lysosomal enzymes and the mannose 6-phosphate receptor. *Histochem J* **20**: 41-51, 1988.
WOO LL, PUROHIT A, MALINI B, REED MJ, POTTER BV: Potent active site-directed inhibition of steroid sulphatase by tricyclic coumarin-based sulphamates. *Chem Biol* **7**: 773-791, 2000.
WU FS, GIBBS TT, FARB DH: Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D-aspartate receptor. *Mol Pharmacol* **40**: 333-336, 1991.
WU FS, CHEN SC: Mechanism underlying the effect of pregnenolone sulfate on the kainate-induced current in cultured chick spinal cord neurons. *Neurosci Lett* **222**: 79-82, 1997.
XU XX, LAMBETH JD: Cholesterol sulfate is a naturally occurring inhibitor of steroidogenesis in isolated rat adrenal mitochondria. *J Biol Chem* **264**: 7222-7227, 1989.
YANASE T, FUKAHORI M, TANIGUCHI S, NISHI Y, SAKAI Y, TAKAYANAGI R, HAJI M, NAWATA H: Serum dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) in Alzheimer's disease and in cerebrovascular dementia. *Endocr J* **43**: 119-123, 1996.
YEN PH, ALLEN E, MARSH B, MOHANDAS T, WANG N, TAGGART RT, SHAPIRO LJ: Cloning and expression of steroid sulfatase cDNA and the frequent occurrence of deletions in STS deficiency: implications for X-Y interchange. *Cell* **49**: 443-454, 1987.
YOO A, HARRIS J, DUBROVSKY B: Dose-response study of dehydroepiandrosterone sulfate on dentate gyrus long-term potentiation. *Exp Neurol* **137**: 151-156, 1996.
ZHANG M, LIU P, DWYER NK, CHRISTENSON LK, FUJIMOTO T, MARTINEZ F, COMLY M, HANOVER JA, BLANCHETTE-MACKIE EJ, STRAUSS JF, 3RD: MLN64 mediates mobilization of lysosomal cholesterol to steroidogenic mitochondria. *J Biol Chem* **277**: 33300-33310, 2002.
ZORUMSKI CF, MENNERICK S, ISENBERG KE, COVEY DF: Potential clinical uses of neuroactive steroids. *Curr Opin Investig Drugs* **1**: 360-369, 2000.

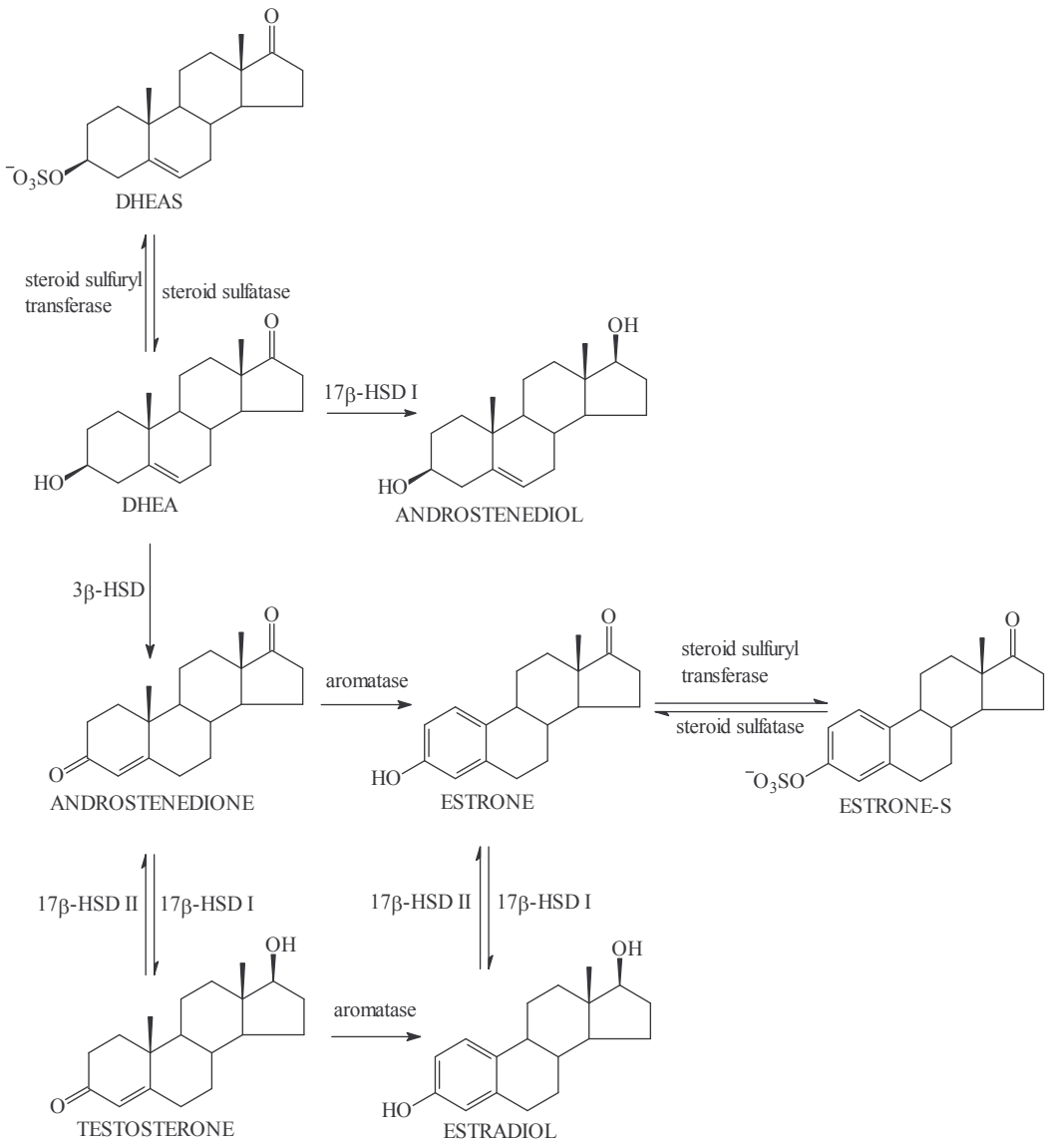

Figure 1.

Steroid hormone metabolism and involved enzymes emphasizing the role of STS in breast cancer tissue. Abbreviations used: 3β-HSD (3β-hydroxysteroid dehydrogenase/$\Delta^{5,4}$-isomerase), 17β-HSD I/II (17β-hydroxysteroid dehydrogenase, type I/II), DHEA (dehydroepiandrosteone), DHEAS (dehydroepiandrosterone sulfate), ESTRONE-S (estrone sulfate).