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Review

The role of sulfated steroid hormones in reproductive processes☆

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ABSTRACT

Sulfated steroid hormones, such as dehydroepiandrosterone sulfate or estrone-3-sulfate, have long been regarded as inactive metabolites as they cannot activate classical steroid receptors. Some of them are present in the blood circulation at quite high concentrations, but generally sulfated steroids exhibit low membrane permeation due to their hydrophilic properties. However, sulfated steroid hormones can actively be imported into specific target cells *via* uptake carriers, such as the sodium-dependent organic anion transporter SOAT, and, after hydrolysis by the steroid sulfatase (so-called sulfatase pathway), contribute to the overall regulation of steroid responsive organs.

To investigate the biological significance of sulfated steroid hormones for reproductive processes in humans and animals, the research group "Sulfated Steroids in Reproduction" was established by the German Research Foundation DFG (FOR1369). Projects of this group deal with transport of sulfated steroids, sulfation of free steroids, desulfation by the steroid sulfatase, effects of sulfated steroids on steroid biosynthesis and membrane receptors as well as MS-based profiling of sulfated steroids in biological samples. This review and concept paper presents key findings from all these projects and provides a broad overview over the current research on sulfated steroid hormones in the field of reproduction.

Contents

Abbreviations: AR, androgen receptor; BTB, blood-testis barrier; DHEAS, dehydroepiandrosterone sulfate; E1, estrone; E2, estradiol; E3, estriol; E1S, estrone-3-sulfate; ER, estrogen receptor; FF, follicular fluid; GC, gas chromatography; GnRH, gonadotropin releasing hormone; hCG, human chorionic gonadotropin; HSD, hydroxysteroid dehydrogenase; LC, liquid chromatography; MS, mass spectrometry; NTCP, Na^+/taurocholate cotransporting polypeptide; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; PREGS, pregnenolone sulfate; RXLI, recessive X-linked ichthyosis; SOAT, sodium-dependent organic anion transporter; STS, steroid sulfatase; SULT, sulfotransferase; TS, testosterone sulfate; TJ, tight junctions.

☆ Concept and review paper of the DFG research group FOR1369 "Sulfated Steroids in Reproduction".

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1. Steroid sulfation and desulfation in reproduction

In man and domestic animals virtually all processes of life are controlled by the nervous and/or the endocrine system. Depending on the organ/cell, the type of neurotransmitters, and the respective endocrine factors, these systems may be distinctly apart or show immediate interactions, for example in the central nervous system, where a number of endocrine factors belonging to the steroid hormone family are known to immediately interact with neural tissue.

To date the term "endocrine factor" also accounts for substances exhibiting paracrine, autocrine and intracrine mechanisms, it comprises the classical hormonal factors, *e.g.* steroid hormones or gonadotropins, but also accounts for cytokines, prostaglandins and a vast variety of growth factors.

Following their detection and identification of about seven decades ago [18,3,19,84], a large amount of information on sex steroids has piled up in respect to production, secretion, metabolism and the mechanisms of action on the cellular and molecular level. This also accounts for their interaction with classic nuclear receptors, DNA binding of ligand activated steroid receptors involving numerous cofactors and the initiation of transcription (for review see Ref. [6]) as well as the mechanisms underlying the fast non-genomic actions of steroid hormones (for review see Ref. [167]).

Though there is a common basic principle underlying the mechanisms of action of steroid hormones, evolution has led to a high diversity in respect to the types, production rates and metabolism of steroid hormones involved in the control of biological processes (*e.g.* [26,27]). This particularly relates to their involvement in reproductive processes.

The classical dogma is that steroid hormones must be available in an unbound, free form in order to interact with the respective receptor and to initiate a biological response. Steroid glucuronides and sulfo-conjugates, which are predominantly formed in the liver or kidney, for long were generally considered as biologically inactive metabolites intended for elimination [51,147,148,69,60,23]. However, already in 1976 Hoffmann et al. [56] described that in pregnant cows large amounts of estrone sulfate (E1S) represent secretory products of the placenta. This also accounts for small ruminants [158,61], the pig [117], the horse [57], camelids [1] and other ungulate species like *e.g.* the reindeer [119]. Similar in the stallion [58] and boar [25] the testes secrete large amounts of conjugated estrogens, in particular E1S. Moreover, the production of significant amounts of E1S has been observed in the ovaries of pregnant mares [28,57] and mares at estrus [82] but not in ovaries of other domestic mammalian species. Though there is ample evidence on the role of estrogens in reproduction, so far no function could be attributed to conjugated estrogens being immediate secretory products of endocrine glands/tissues.

The discovery of the co-localization of estrogen receptors (ER), steroid sulfatase (STS) and estrogen sulfotransferase (SULT) in the same tissue sheds new light on this situation [133] and gives rise to new hypotheses and speculations on the role of conjugated estrogens in reproduction and reproductive diseases, *e.g.* mammary gland tumors [115]. Increasing evidence has come up during the last years that hydrolysis of sulfo-conjugated estrone and dehydroepiandrosterone catalyzed by STS is an important alternative source of precursors for the local supply of estrogens and androgens, respectively [137,100]. In humans STS has recently been identified as a valuable drug target for estrogen and androgen deprivation therapies in hormonal diseases [146]. Thus, in addition to the provision of steroid hormones by the secretory activity of a given cell or gland, a second system controlling the availability of biologically active steroids on the cellular level might be established due to the expression of STS and/or SULT in certain organs, like the placenta [133], mammary gland [62], ovary [82,28,57], brain [109], or the testis [91].

The existence of such a system, however, would also require that sulfated steroids penetrate the plasma membrane of a target cell in order to get hydrolyzed and to become biologically active. The fact that – other than free steroids – conjugated steroids will not passively pass the lipid cell membrane barrier, questioned the existence of such a secondary local regulatory system. This situation has changed with the discovery of membrane uptake carriers for sulfated steroids such as the sodium dependent organic anion transporter (SOAT). SOAT has been shown to have high substrate specificity for sulfated steroids and is highly expressed in reproductive tissues such as testis and placenta [41,43,36,45,136].

The research group "Sulfated Steroids in Reproduction" of the German Research Foundation DFG focuses on this hypothesis of a local regulatory system concerning the provision of biologically active steroids by using sulfated steroids as precursors or by targeting biological activity through sulfation of free steroids. Fig. 1 depicts in a schematic way the basic concept of our research group. This concept relates to the observation that sulfation undoubtedly abolishes steroid hormone binding to their receptors, but that there is a species-specific production of sulfated steroid hormones, particularly of dehydroepiandrosterone sulfate (DHEAS) and E1S, by reproductive organs of hitherto unresolved biological meaning. Species exhibiting such high levels of sulfated steroid hormones include man and pig. The majority of projects are related to these two species and deal with target tissues for sulfated steroids and tissues as sources of their formation such as the testis and placenta. As the endogenous synthesis of steroid hormones by hormone competent cells competes with the recruitment of steroid hormones by desulfation, the balance between endogenous highly energy consuming synthesis of hormones from cholesterol and the rapid liberation of active hormones from their inactive sulfated precursors constitute a new regulation pathway. All reproductive tissues are subjected to feedback regulation *via* the brain, so sulfated neurosteroids may be important for the neuroendocrine control at the level of the anterior hypothalamus as well.

2. Transport of sulfated steroid hormones in the testis: the role of the sodium-dependent organic anion transporter SOAT

The importance of testicular testosterone production for spermatogenesis is well established. The prime target sites for androgens in the testis, as shown by the expression of the androgen