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REGULATORY INTERACTIONS FOR THE CONTROL OF STEROID SULFATE METABOLISM

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SUMMARY

The 5-ene-steroid-3β-monosulfates (SMS) are accepted as potential intermediates in androgen biosynthesis *in vivo*. Evidence from this laboratory and others are reviewed to support the premise that despite low conversions, the physiological significance of the regulation of SMS cleavage is important for androgen biosynthesis primarily at the target site of responsive tissue. Steroid sulfatase enzyme, regardless of tissue source in mammals, appears to be a monomeric unit having a steroid sulfate binding-site and a second regulatory steroid binding-site. Monomer or its aggregates intimately associated with particulate cell membrane implicate both transport and metabolism of steroid hormones. A model is presented in which SMS (substrates) contribute to androgen biosynthesis through hormonally (steroid allosteric modifiers and inhibitors) regulated cleavages at the cell membrane by steroid sulfatase enzyme at the site of hormone (steroid product) utilization. This is suggested to occur at the blood–organ barrier in target tissues sensitive to androgens, and in particular as a means of regulating the level of androgens within the seminiferous tubule.

INTRODUCTION

The 5-ene-steroid-3β-monosulfates (SMS) as potential intermediates in androgen biosynthesis have been established through *in vivo* demonstrations of the interconvertibility of dehydroepiandrosterone (D) and its sulfate (DS), [1, 2] the direct conversion of pregnenolone sulfate (PS) to DS [3] and the conversion of DS to testosterone in amounts estimated from 3 to 12% [4–6]. However, the *in vivo* sites of these metabolic conversions and their regulation have not been clearly established. Despite the obvious involvement of steroid sulfatase activity, precise definition of the enzyme system and its coherent participation in steroid biosynthesis has been elusive. A persistent yet not entirely convincing model for SMS utilization postulates a low-capacity auxiliary pathway to augment the output from organs which secrete steroid hormones. One major difficulty amongst many is reconciling the redundancy inherent in this scheme. An entirely different concept is now introduced, in which this aspect of steroidogenesis is invoked specifically for target sites.

In this presentation, previous findings from this laboratory and others are reviewed and used to develop a more precise model. In this model, SMS contribute to androgen biosynthesis through hormonally regulated cleavage at the cell membrane by steroid sulfatase enzyme at the site of hormone utilization in target tissues sensitive to androgens.

Steroid sulfatase

Occurrence. Mammalian steroid sulfatase (Sterol sulfate sulfohydrolase, EC. 3.1.6.2), first observed in microsomal preparations of ox and rat liver [7, 8], is found in liver, testis, ovary and adrenal [9], placenta [10], prostate [11], skin [12] and many other tissues (brain, fetal lung and viscera) so as to be virtually ubiquitous in vanishingly small quantities.

Baillie and Griffiths[13] demonstrated a histochemical identification of 3β-hydroxysteroid dehydrogenase activity in mouse testes indicating a difference in substrate utilization of steroid sulfates and unconjugated steroids. DS was not utilized histochemically by Leydig cells, but minute diformazan crystals were deposited next to the sperm heads in the seminiferous epithelium from the end of the third week of postnatal life on. The possibility that seminiferous epithelium constitutes a target organ for DS was suggested. The participation of mammalian steroid sulfatase is implicit.

A recent biochemical study by Kawano *et al.*[14] demonstrated that human testicular steroid sulfatase is concentrated principally if not entirely in seminiferous tubules rather than interstitial tissue. Moreover, comparisons drawn with rat testis tissue suggest that Leydig cells are the major site of androgen production, as reported earlier by Hall *et al.*[15] and Christensen and Mason[16].

It now seems obvious that in order to appreciate the significance of the role of the SMS in steroid hormone biosynthesis, a clear distinction must be made between the Leydig cells of the testis, which are the endocrine organ producing androgens in response to gonadotropins, and the seminiferous tubules, which are target tissue for androgen action. Although other steroid metabolizing capabilities are observed for the seminiferous tubules, it appears that steroid sulfatase activity may be exclusively endowed to this and all other target tissues. Such metabolic capabilities in target tissues would ensure a precisely controlled environment of active hormones and also utilize a wide range of hormone precursors.

Table 1. The cleavage of steroid sulfates as modified by the addition of steroid compounds*

Substrate cleaved	Apparent K_M moles/liter	Added steroid	Apparent K_I moles/liter
Pregnenolone sulfate	$0{\cdot}3 \times 10^{-5}$	5-Pregnene-3β,20α-diol	$0{\cdot}3 \times 10^{-5}$
		Pregnenolone	$0{\cdot}8 \times 10^{-5}$
		Testosterone	$0{\cdot}8 \times 10^{-5}$
		17α-Hydroxypregnenolone	$1{\cdot}4 \times 10^{-5}$
		Dehydroepiandrosterone sulfate	not detected
Androst-5-ene-17β-ol-3β-sulfate	$0{\cdot}5 \times 10^{-5}$	5-Androstene-3β,17β-diol	$0{\cdot}6 \times 10^{-5}$
		Testosterone	$0{\cdot}8 \times 10^{-5}$
		Dehydroepiandrosterone sulfate	$1{\cdot}5 \times 10^{-5}$
Dehydroepiandrosterone sulfate	1×10^{-5}	Dehydroepiandrosterone	$0{\cdot}75 \times 10^{-5}$
		Testosterone	$0{\cdot}7 \times 10^{-5}$
17β-Estradiol sulfate	$1{\cdot}2 \times 10^{-5}$	Estradiol-17β	$1{\cdot}6 \times 10^{-5}$
		Testosterone	$1{\cdot}3 \times 10^{-5}$
		Estrone sulfate	$2{\cdot}8 \times 10^{-5}$
Estrone sulfate	2×10^{-5}	Testosterone	$0{\cdot}8 \times 10^{-5}$
		Estrone	$2{\cdot}2 \times 10^{-5}$
		Dehydroepiandrosterone sulfate	$1{\cdot}5 \times 10^{-5}$
17β-Acetyl-5-androstene-3β-sulfate	3×10^{-5}	5-Androstene-3β,17β-diol	$0{\cdot}1 \times 10^{-5}$
		Testosterone	$0{\cdot}6 \times 10^{-5}$
Cholesteryl sulfate	5×10^{-5}	Testosterone	$0{\cdot}7 \times 10^{-5}$
		Cholesterol	$1{\cdot}2 \times 10^{-5}$
		Pregnenolone sulfate	$0{\cdot}4 \times 10^{-5}$
5-Pregnen-20α-ol-3β-sulfate		Cleavage at 1 h at concn $2{\cdot}3 \times 10^{-6}$ m/l	
Cyclohexyl sulfate		No cleavage at 3 h at concn 5×10^{-6} m/l	

* Data from Notation A. D. and Ungar F.: *Steroids* **14** (1969) 151–159.

Specificity and kinetic behavior. Notation and Ungar[17] demonstrated the inhibitory effect of D and also of testosterone (T) on the *in vitro* cleavage of DS by rat testis tissue, thus introducing a regulatory aspect to the cleavage of SMS. Subsequent kinetic studies [18, 19] are summarized as substrate specificities and inhibitor actions (Table 1). Substrate specificities were ranked by increasing K_m values. PS is the most efficient substrate, followed by the 3-sulfates of 5-androstene-3β,17β-diol (5-ene-A-diol), D, 17β-estradiol (E_2), estrone (E_1) and cholesterol. Virtually no cleavage was observed for the 3β-sulfate of 5-pregnene-3β,20α-diol (5-ene-P-diol). The preferred PS cleavage, on the other hand, is inhibited most efficiently by 5-ene-P-diol. These findings are consistent with the concept of a single steroid sulfatase enzyme to which either substrates or unconjugated steroids bind, to an extent partially determined by substituents at C-17 as well as the conformation at C-5 and C-3.

Confirmation and extension of these findings by Payne *et al.*[20] introduced the possibility that mammalian steroid sulfatase may be an allosteric enzyme. Steroid sulfatase was suggested to have two steroid binding-sites, one where steroid sulfates are cleaved and a second which exerts regulatory actions. Furthermore, if this enzyme exists in a high molecular weight polymeric form capable of structural flexibility, then certain unusual kinetics of cleavage inhibition would be explained in terms of a conformational change which occurs at a critical concentration of a designated inhibitor.

Townsley *et al.*[21, 22] described the kinetic characterization of human placental steroid sulfatase activity, which accentuates the similarities with testicular steroid sulfatase already described and provides indication of readily identifiable differences.

Physical properties. Steroid sulfatase is apparently an integral part of rat liver microsomal membrane. Burstein[23] attempted to solubilize steroid sulfatase through digestion with phospholipase A (heat treated snake venom) and further purification. This resulted in a material with an approximate molecular weight of 600,000 which was enriched in steroid sulfatase activity but tended to form insoluble aggregates.

Bleau *et al.*[24] determined a molecular weight of approximately 23,000 for what is believed to be the monomeric form of rat liver microsomal steroid sulfatase on polyacrylamide gel electrophoresis in the presence of sodium lauryl sulfate. Likewise, many stable forms of the enzyme were indicated, ranging from the monomer to an undesignated upper limit which did not exclude 600,000.

Considerations involving membrane barriers, steroid transport—metabolism interactions, and allosteric steroid sulfatase

Fawcett *et al.*[25] have observed that man, monkey, ram and, to a large extent, rat have comparable tes-

ticular morphology. In these and other species, high permeability of the intertubular vessels would ensure continuous access of steroid-binding proteins from the blood to the surface of the Leydig cells and tubules, but protein-bound androgens would be excluded by the permeability barrier in the wall of the tubule. Van Doorn *et al.*[26] noted a possible influence of plasma proteins on the transport and uptake of certain radiolabeled androgens in rabbit testis tubule. Indeed, there appears to be a barrier to androgen in ram testis where Setchell[27] measured a testosterone level of 4·9 μg/100 ml of testicular lymph, as compared with 2·78 μg/100 ml of rete testis fluid.

Dym[28] describes the blood-testis barrier as being made up of Sertoli cells and communicating membrane junctions between neighboring Sertoli cells. A steroidogenic function for the Sertoli cells could be rejected, on the basis that rat seminiferous tubules *in vitro* as studied by Hall *et al.*[15] are unable to convert cholesterol-7α-[^{3}H] to androgen. However, Lacy and Pettitt[29] reviewed and confirmed evidence to establish that in rat testis tubule, as distinct from interstitium, there are two main pathways for steroidogenesis from progesterone precursor. By a pathway common to interstitium but quantitatively less important, the formation of testosterone by Sertoli cells is primarily concerned with evoking a local reaction within the confines of the tubule. The second pathway leads to various hydroxylated and/or reduced pregnanes/pregnenes, such as 20α-dihydroprogesterone, with uncertain biological implications. In man, both isolated tubule and interstitial tissue elaborated testosterone from added precursors through pathways suggesting greater involvement of 5-ene-3β-hydroxysteroid intermediates. Payne and Mason[30] indicated this to be a natural consequence of the involvement of steroid sulfates in androgen biosynthesis. First, the gonadotropin-sensitive conversion of cholesterol to pregnenolone (and/or their respective conjugates) is the exclusive property of the steroid hormone-secreting endocrine organ, such as the Leydig cell. However, subsequent conversions of pregnenolone to steroid hormones by unconjugated and conjugated pathways are apparently free of direct gonadotropin control. This second level of metabolic conversions includes all of the ones described above for the Sertoli cell. Formation of steroid hormones in small quantities outside of the endocrine organs, previously considered non-specific by metabolism in the peripheral system, now fits into the role of a regulated hormone biosynthesis at specific target organ sites and is incorporated into the model.

The limitations on the steroidogenic capabilities of Sertoli cells, Sertoli cell involvement in the blood-testis barrier, and the histochemical demonstration of steroid sulfate metabolism near the head of the developing sperm favor the probability that testicular steroid sulfatase, located exclusively in the tubule, is an integral part of the membrane within the blood-testis barrier and a requisite enzymatic component in the transport and regulation of steroidogenesis in the Sertoli cell.

Liver, with its interface for the peripheral and the enterohepatic circulatory system; kidney, with its function of urinary steroid excretion; and placenta, with its barrier isolating the fetus; are all obvious examples of the importance of the transport of steroids and their conjugates. Bleau *et al.*[31] have shown that liver steroid sulfatase in the presence of sterol sulfates and/or their analogs loses its ability to cleave a more preferred substrate DS while simultaneously altering the kinetics of cholesterol sulfate cleavage. Kidney sulfatase which does not cleave DS was observed by Zuckerman and Hagerman[32] to cleave estrogen-3-sulfates, but other steroid sulfates were not tested. Bovine adrenal microsomal steroid sulfatase was observed by Notation and Ungar[33] to cleave the 3-sulfate derivatives of cholesterol, P, and E_1, but not D or 5-ene-A-diol. The inability to cleave DS should not be used alone to indicate the absence of steroid sulfatase activity. The selective shift to an otherwise less preferred CS cleavage supports a yet unidentified transport role for CS, as discussed by Hochberg *et al.*[34]. More importantly, this lends further emphasis to the probability that mammalian steroid sulfatase, by its allosteric activity at the cell membrane participates in specific and controlled activity regulating the formation, transport and biological action of steroid hormones.

In vivo *evidence to indicate multiple control mechanisms of steroid sulfatase enzyme*

Laatikainen *et al.*[35] presented data on the secretion of neutral steroid sulfates by human testis (Table 2) but did not explain instances where the level of DS was lower in spermatic vein than in peripheral vein blood plasma. One may note that in these cases the concentration of 5-ene-pregnanediol-3-monosulfate (5-ene-P-diol-S) is invariably lower, by 30 to 40%, in fact, when compared with cases in which no differential in DS levels is observed. Conversely, elevation of the concentrations of these two sulfates appear to be correlated also. Since PS appears to be secreted by the testis in all cases, one may conclude that it is synthesized *de novo* in the testis but not necessarily metabolized. On the other hand, DS appears to be abundantly supplied to the testis and can be diminished or augmented in a manner seemingly related to the disposition of 5-ene-P-diol-S.

The apparently preferred formation of hydroxylated and/or reduced pregnanes/pregnenes by the Sertoli cell [29] may well be favored under conditions where both unconjugated androgen and P have reached or exceeded a level of sufficiency. If so, the SMS participation in androgen biosynthesis would be redundant. The effectiveness of 5-ene-P-diol as a steroid sulfatase inhibitor and the unsuitability of 5-ene-P-diol-S as a substrate [19] may indeed focus on the shut-off for all steroid sulfate cleavage as far as androgen biosynthesis is concerned (Fig. 1). Thus, special importance is assigned to pregnenolone and

Table 2. Concentrations of steroid sulfates in peripheral vein (Pe) and spermatic vein (Sp) blood plasma (values expressed as μg of the free steroid in 100 ml of plasma)*

		Dehydroepiandrosterone	5-Pregnene-3β,20α-diol
VJ	Pe	103	31
	Sp	83	24
VP	Pe	124	23
	Sp	102	17
MP	Pe	28	14
	Sp	23	10
KP	Pe	16	4·9
	Sp	13	3·4
KS	Pe	62	23
	Sp	87	32
KJ	Pe	30	9·0
	Sp	41	11·0
EM	Pe	53	16
	Sp	78	21
NH	Pe	13	9·7
	Sp	24	9·0
ER	Pe	53	19
	Sp	52	18
GT	Pe	29	11
	Sp(L)	40	12
	Pe	31	10
	Sp(R)	31	8·6
EN	Pe	21	6·1
	Sp	20	5·2

* Data obtained from "Secretion of Neutral Steroid Sulfates by the Human Testis" Laatikainen T., Laitinen E. A. and Vihko R.: *J. clin. Endocr. Metab.* **29** (1969) 219.

probably progesterone as a likely on–off locus for SMS contribution to hormone biosynthesis at the target site. When the on–off mechanism is not fully controlling the steroid sulfatase enzyme, the SMS cleavages are no doubt influenced by a wide range of simultaneous effects, particularly substrate specificities, inhibitor actions, and other factors. If plasma concentrations accurately depict the relative amounts of steroid sulfate [35] available to the testis tubule, then DS is quantitatively the most important SMS available for androgen biosynthesis.

As in the case of P and PS, D and DS can give rise to some metabolites which are not precursors of testosterone. However, other of these C_{19} metabolites arise also from the metabolism of testosterone. A further complication is that some of these metabolites, most notably dihydrotestosterone, have been implicated as primary agents responsible for androgenic activity. The unusual kinetics observed for the cleavage of steroid sulfates in the presence of various unconjugated 3,17-dioxygenated-5α-androstanes may indicate the locus of allosteric control with an implicit capability of directing substrate specificity. The capacity to cleave 3β-sulfoxy-5α-androstane derivatives may be a mechanism to further supply modifiers for allosteric control of sulfate cleavage and/or provide either a precursor or an active agent for eliciting androgenic responses in tissue.

Steroid sulfatase participation in mechanisms of steroid hormone action

An emerging concept of transport and steroid

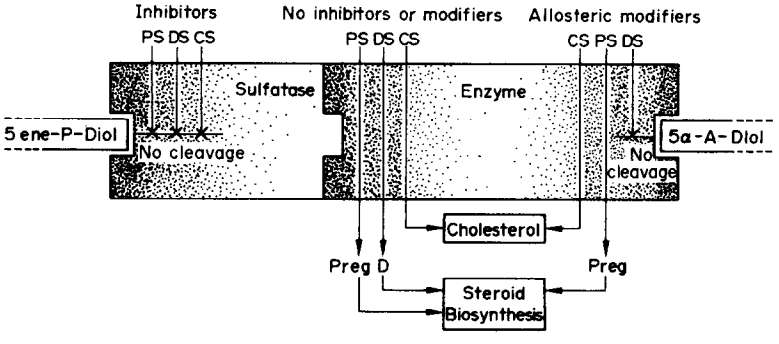

Fig. 1. Components of membrane–sulfatase regulation. (First approximation.)

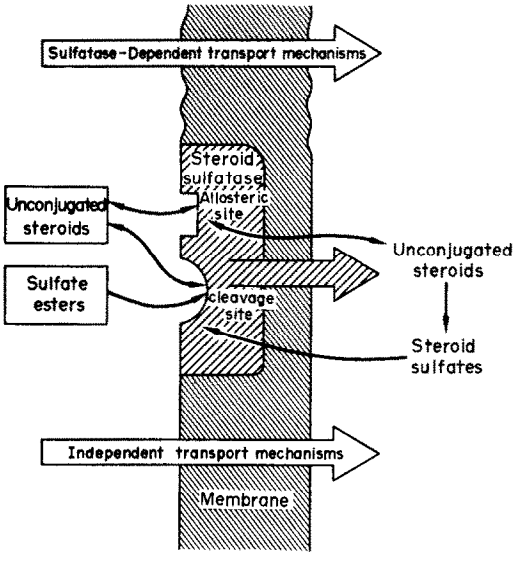

Fig. 2. A model for steroid sulfatase–membrane activity at the target site.

metabolism intimately associated with hormonal action can be formulated from CS–DS-steroid sulfatase interaction. Initial associations of steroid sulfatase with androgen-responsive tissues have been reviewed in terms of regulating steroid hormone transport across membrane barriers, as one means to control levels of active hormone at the target site (Fig. 2). More specifically, each blood–organ barrier containing steroid sulfatase may be considered individually to be a target site.

Therefore, steroid sulfatase activity is an integral part of steroid hormone action because changes in enzyme activity are observed when known steroid hormones bind to steroid-specific regulatory sites on this enzyme. The cleavage of CS which is preferred over DS and other steroid sulfates under certain conditions may indicate that steroid sulfatase activity is necessary in action to cause membrane perturbations even when SMS are not required for steroidogenesis. The enzyme functioning in this manner is under allosteric control, presumably responding to bound steroid modifiers. Transport across this membrane barrier as a mechanism of hormone action appears to be oriented toward regulating the levels of unconjugated steroid within the cell. Obviously, this must occur cooperatively with other elements of transport, most notably the steroid carrier-proteins in the inter- and intra-cellular fluids. This proposed level of hormone response may then be concerted with the regulated exposure of steroid hormones to the cytoplasmic and nuclear receptors which activate the classical hormonal responses known to occur in target tissues. It is worth noting that in this model transport across the membrane as a mechanism of hormone action is not restricted only to the transport of steroids, but may include other molecules as well.

Another regulatory influence to consider is the formation of steroid sulfates. The study of steroid sulfokinase is incomplete, but its presence as a soluble intracellular enzyme must be at least as widespread as its particulate opposite number, steroid sulfatase. A ubiquitous steroid sulfokinase could efficiently scavenge steroids not immediately in use (bound to protein) and "inactivate" them for storage and/or excretion. The short biological half-life of unconjugated steroids in peripheral circulation emphasizes the moment-by-moment regulation of their use. The formation of steroid sulfates and their retention in the peripheral blood as a reservoir function described by Notation and Ungar[17] provides virtually every tissue with a uniformly large supply of SMS precursors for on-site regulation of androgen levels. Quantitatively this reservoir is virtually maintained by CS supplied by the liver and DS supplied by the adrenals. Input from gonads and target organs, with consideration of other metabolic and transport modifications, exerts a regulatory influence on the composition of this steroid sulfate reservoir. The utilization of these SMS would appear to be under direct steroid hormone influence [36].

Steroid sulfatase as a site for steroid hormone action thus provides some basis for identifying possible target response. Furthermore, the suspected biological activity of various steroid metabolites might be partially systematized by quantitating their kinetic effect on steroid sulfate cleavages. The action of steroid sulfatase in contributing androgens for hormonal action is responsive to estrogen substrates and inhibitors. The contribution of androgens as precursors of estrogens and/or the cleavage of estrogen sulfates could eventually represent an important interface for synergism between androgen and estrogen hormonal activities. A successful realization of these applications would provide an as yet unexploited approach to elucidating and controlling steroid hormone action.

The following abbreviations and trivial names are used: dehydroepiandrosterone (D), 3β-hydroxy-5-androstene-17-one; pregnenolone (P), 3β-hydroxy-5-pregnen-20-one; epiandrosterone, 3β-hydroxy-5α-androstan-17-one; estrone (E_1), 3-hydroxy-1,3,5(10)-estratriene-17-one; estradiol (E_2), 1,3,5(10)-estratriene-3,17β-diol.

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