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REVIEW

3α-Hydroxysteroid Dehydrogenase in Animal and Human Tissues

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Abstract—This review analyzes data on the biological role of 3α-hydroxysteroid dehydrogenase (3α-HSD) in animal and human tissues and describes its main characteristics, mechanism of action, and regulation of activity. Based on published data, a scheme for the actions of androgen, progestin, and glucocorticoids involving the participation of 3α-HSD is proposed. According to this scheme, in the mechanism of steroid action 3α-HSD not only regulates the concentration of the main effector androgen, 5α-dihydrotestosterone, in target cells, but also switches androgen, progestin, and glucocorticosteroid genomic activity to non-genomic activity.

Key words: 3α-hydroxysteroid dehydrogenase, structure, mechanism of action, biological role

Hydroxysteroid dehydrogenases (hydroxysteroid oxidoreductases, HSD) belong to a broad aldoketoreductase (AKR) superfamily [1] and are involved in reactions both in steroid biosynthesis and metabolism in endocrine and exocrine tissues. However, their role in mechanisms of steroid action is yet unclear. Of special interest among these enzymes are 3-HSDs that catalyze redox reactions of the C3 keto(hydroxy)-group in the A ring of many 5α- or 5β-reduced steroids. There are two 3-HSDs known so far, each of them demonstrating individual specific activity resulting in the production of two steroid types, i.e., 3α-hydroxysteroids and 3β-hydroxysteroids.

In the 1990s there was a significant shift in the interest of investigators to steroid production in tissues where 3α-HSD and 3β-HSD make a great contribution: the investigators focused on individual enzymes participating in these processes rather than on comparison of steroid metabolism patterns in different tissues. There are several reasons to account for this shift, and we consider two of them most important.

First, the classical approach to the study of the role of steroid metabolism in the body based on comparison of enzyme activities in steroid production processes at the tissue, cellular, or subcellular fraction levels was to a certain degree exhausted about a decade ago. These studies were extremely fruitful as to the role of steroid production in different animal and human tissues. Nevertheless, there still were many questions to be answered, and new approaches were needed to give the answers.

Second, progress in molecular biology made possible the structural study of many enzymes contributing to steroid production because codes of their encoding DNA and respective amino acid sequences were established. Due to the availability of significant amounts of native and mutant enzyme forms, not only the exact mechanisms of action and three-dimension structures of several enzymes were determined but also detailed study of their specific features was continued.

Very impressive results were obtained for an important and interesting HSD enzyme participating in steroid production, i.e., 3α-hydroxysteroid:NAD(P) oxidoreductase, EC 1.1.1.213 (3α-HSD), which is found in many animal and human tissues.

This review discusses 3α-HSD features and mechanism of action as well as results of its structural study. We attempted to evaluate the role of 3α-HSD in mechanisms of action of some 3α-hydroxysteroids in animal and human steroid target tissues.

The following important circumstance was taken into account when writing this report. As mentioned above, both 3α- and 3β-hydroxysteroids are produced from a 3-keto-5α-reduced steroid (most often this is 5α-dihydrotestosterone, DHT) that is normally used to measure 3-HSD *in vitro* activity. These epimers are diffi-

Abbreviations: HSD) hydroxysteroid dehydrogenase; AKR) aldoketoreductase; 5α-R) 5α-reductase; DHT) 5α-dihydrotestosterone; NSAID) nonspecific non-steroid anti-inflammatory drugs; GABA) gamma-aminobutyric acid.

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cult to separate [2]. Therefore, this review discusses only those studies in which the 3α- and 3β-HSD activities were clearly distinct.

ENZYME PROPERTIES, MECHANISM OF ACTION, AND REGULATION OF ACTIVITY

The 3α-HSD activity converting dihydrocorticosteroid into tetrahydrocorticosteroid was first described for rat liver in 1956 (cited from [3]). Later the enzyme was found in most peripheral tissues and in gonads of animals and humans as well as in several human tumors and tumor cell lines. Of most interest as to 3α-HSD are brain, prostate, liver, skin, lung, kidney and gonads, i.e., the principal target sites for steroid hormones. 3α-HSD activity varies considerably in different tissues, though the reason for this variability is unclear.

As concerns steroid hormones, 3α-HSD acts as reductase-dehydrogenase [3] in the reversible reaction

$$\text{3-ketogroup} \leftrightarrow \text{3}\alpha\text{-hydroxygroup}$$

in C19-, C21-, and C24-steroids reduced with respect to the A ring. Some authors [4, 5] believe that A/B ring conformation is of no importance, while in the opinion of others [6, 7] this may have an effect on the rate of reaction with 3α-HSD. 3α-HSD is also active in relation to many non-steroids (see below), and in this case the enzyme acts mainly as a dehydrogenase [3].

There are two subcellular forms of 3α-HSD found in animal and human tissues, i.e., microsomal and soluble (cytoplasmic) forms, the latter having several isoforms in monkeys and humans, though these data are equivocal [1, 8-15] (see below). The cytoplasmic and microsomal 3α-HSD activities can vary considerably even in different parts of the same organ [16] as well as changing during ontogenesis [17] or in pathologies [18]. This feature may be due to alteration in activity of only one subcellular form of the enzyme, as demonstrated for brains from rats of different age [19], or to preponderance in activity of one of the forms in relation to a certain steroid group [20].

We think that differentiation in enzymatic activity subcellular distribution is of much interest. For instance, cytoplasmic 3α-HSD activity is greater than microsomal 3α-HSD in rat liver and skin, while in liver and prostate of man and dog (in which microsomal forms seem to play a more important role) microsomal 3α-HSD activity is about one order of magnitude greater than the cytoplasmic activity [8, 18, 21].

The optimal pH for the reductase reaction is 6.0-7.4 for soluble and 7.0-8.0 for microsomal 3α-HSD [22]. Soluble 3α-HSD reductase and dehydrogenase activities also differ in optimal pH: the pK_a for reductase activity is 5.6, but it is 8.9 for dehydrogenase activity [3].

Different 3α-HSD reductase and dehydrogenase activities can be found in the same tissue with reductase activity predominating in most cases [22], though dehydrogenase activity may predominate in some pathologies [23, 24]. This suggests that in some tissues there are (or may change) conditions under which the reaction with 3α-HSD participation preferably proceeds in one direction, as in human, dog, and rat prostate [3, 24-27], or one or the other activity is found due to a preponderance of the respective enzyme isoform.

It was supposed in early studies that 3α-HSD was equally active in the presence of NAD(H) and NADP(H). However, later studies of soluble 3α-HSD in rat liver demonstrated that enzyme affinity to the cofactors varied considerably: the K_d value of apoenzyme binding to the cofactors was 165 μmol/liter for NADH against 190 nmol/liter for NADPH [3]. This means that catalysis with the participation of 3α-HSD is more favored in the presence of NADPH, though many studies of the mechanism of action of the enzyme were performed using NADH [3, 28].

The cofactor saturating concentrations are 0.2 to 0.6 mM for all 3α-HSD forms. The apparent K_m values are 0.2 to 10.0 μM for dehydrogenase activity [10, 11, 17, 29] while being 5-10-fold lower for reductase activity [1].

Bivalent metal (Ca, Mg, Zn) ions inhibit 3α-HSD activity [30].

Soluble 3α-HSD activity does not seem to depend upon temperature and is high even at 0°C, as demonstrated for rat uterus [31], rat Sertoli cells (after 3 h 50% of DHT was converted into 5α-androstane-3α,17β-diol (3α-D)) [32], and rat heart muscle (DHT was almost completely converted into 3α-D) [33].

It is difficult to compare enzyme features even for the same tissue, leaving alone different tissues or organisms, since investigators use different experimental conditions. Historically there has been more intensive study of soluble 3α-HSD from different tissues. Therefore, the review below discusses features of the soluble enzyme unless a specific subcellular form is specified.

As demonstrated for the soluble enzyme from rat liver, the 3α-HSD molecule contains nine SH-groups, though alkylation of only a single SH-group reduces dramatically the enzyme activity [3, 4] (see below).

It seems that there is a single soluble liver 3α-HSD in most animals [1, 5, 14, 15, 34] and four liver isoenzymes with 3α-HSD activity in humans, one of which, 3α-HSD type 1, can be considered specific for human liver (see below for details of 3α-HSD isoforms).

Homogeneous soluble 3α-HSD has been isolated from several human and animal tissues. The enzyme was shown to be a monomeric protein with molecular weight 25-39 kD [1, 8, 10, 11, 14, 19, 34], though an enzyme isoform specific for humans might also occur as a dimer [15].

Rat liver 3α-HSD constitutes about 1% of the total soluble protein [3], and this explains why the soluble

enzyme from rat liver is the best-studied 3α-HSD form [1].

The full amino acid sequence, exact mechanism of action, and three-dimensional structure of 3α-HSD have been established [28]. This was possible due to the rare chance of obtaining crystalline apoenzyme forms and a binary complex with a cofactor (see below) [3, 28]. There has been vast molecular genetic study of 3α-HSD from rat and human liver in comparison with other enzymes from the AKR superfamily. As reported in many publications (see, for example [3]), it was also demonstrated that the amino acid sequence of human liver-specific 3α-HSD is 68-78% identical to the enzyme sequences from rat and rabbit liver [9], but this analysis is beyond the scope of this report.

Rat liver 3α-HSD-encoding DNA contains 966 base pairs as reported by some authors [3] or 1286 as reported by others [35] with an open reading frame for the monomeric protein. The enzyme polypeptide chain contains 322 (rat liver) or 323 (human liver-specific 3α-HSD) amino acid residues with Met at the N-terminus and Glu (rat) or Tyr (human) at the C-terminus [3, 28].

The tertiary structure of the enzyme polypeptide chain (X-ray crystallographic analysis) of the rat liver soluble 3α-HSD is formed by eight alternating α-helix and β-strand structure that form a $(\alpha/\beta)_8$-barrel with β-sites on the internal side of the barrel [3]. The polypeptide chain forms loops A (amino acid residues 22-32) and B (residues 220-227) on the barrel ends.

The substrate-binding site of the polypeptide chain has a cleft shape formed by side groups of ten amino acids, the most important being Leu54, Trp86, Phe128, Phe129 (located in β_2- and β_3-sites) and Trp227 (in loop B) [3]. Cys217 is also of importance for the substrate binding because directed mutagenesis of the enzyme involving substitution of Ala for Cys217 results in a considerable reduction in binary complex affinity to the steroid [3].

The substrate-binding cleft in the 3α-HSD molecule is located so that one of its ends is adjacent to loop A and the other comes close to loop B. It seems to be important for the enzyme activity to have a cleft with variable volume to provide enough room for substrate molecules of different size [3]. This phenomenon partially explains both why different steroid types may act as 3α-HSD substrates and why the enzyme demonstrates polysubstrate activity in relation to non-steroid dihydroxy compounds [3]. Moreover, a steroid can also be bound in the cleft if its molecule is rotated by 180° around its vertical axis and, as a result, the D ring of the steroid molecule comes close to the enzyme active site instead of ring A [3]. It has also been demonstrated that only two human liver 3α-HSD isoenzymes and the rabbit liver enzyme show 17β- or 20α-HSD activities [3, 7, 34, 36] (see also below).

Study of the mechanism of action of 3α-HSD has shown that the reaction of production of 5α-androstane-3α,17β-diol (3α-D) from DHT (reductase activity of the enzyme) can be divided into four stages [1, 21, 22].

At stage one, the cofactor binds to the apoenzyme, which results in a so-called binary complex [3, 13, 37]. The second reaction stage can start only after this complex is formed; it consists of binding to the substrate and results in the production of an intermediate (ternary) complex. Note that the apoenzyme cannot bind with steroid substrates or steroid inhibitors without a cofactor, though it does with non-steroid inhibitors [3]. At stage three the intermediate complex decays into the reaction product and the binary complex, which in turn decays into the cofactor and the apoenzyme. The reaction with the participation of 3α-HSD follows a "bi-bi" pattern in which the cofactor is the first to bind to the apoenzyme to form a binary complex (probably with certain conformational changes in its polypeptide chain that help in the substrate binding) and the last to separate after the reaction ceases [37]. Stages one and four, i.e., cofactor binding and separation, are reaction limiting stages [1].

The interesting ability of the enzyme to retain the stability of the cofactor complex during crystallization was effectively used to study in detail the structure of the binary cofactor–3α-HSD complex from rat liver [3]. In this complex the cofactor was located perpendicularly to the axis of the barrel molecule formed by the polypeptide chain of the enzyme [3]. At least 11 amino acid residues of the polypeptide chain of 3α-HSD form hydrogen bonds with the cofactor in the binary complex. The nicotinamide ring comes very close to the Tyr216 residue and initiates π-interaction [3]. The position of the carboxamide residue of the nicotinamide chain is fixed due to hydrogen bonds between itself and Ser166, Asn167, and Glu190 as well as to a bond between the cofactor 2′-phosphate group and Arg276 of the enzyme. These hydrogen bonds force the β-plane of the nicotinamide ring of the cofactor molecule neighboring the hydrogen that takes part in the reaction 4-(*pro*-R)-hydrogen to turn to the internal surface of the hydrophobic cleft in which substrate binding occurs. Recent direct mutagenesis studies suggest that NADP(H) and NAD(H) may have different strengths of binding to the apoenzyme [38]. The preliminary cofactor binding and further positioning of hydrophobic amino acid residues contributing to the substrate binding are likely to favor to the substrate molecule to occupy a position such that the β-plane of the steroid A ring is oriented towards the β-plane of the cofactor nicotinamide ring. The latter circumstance is of much importance for the stereo-specificity of the reaction.

Elegant experiments with *pro*-R-[4-^{3}H]NADH and *pro*-S-[4-^{3}H]NADH demonstrated that 3α-[^{3}H]O-steroid production under the effect of rat liver 3α-HSD was possible only with the first cofactor [37, 38]. It follows then that the 4-(*pro*-R)-hydrogen from the adenine dinucleotide nicotinamide ring attacks the 3-ketogroup of the substrate steroid from the side of the β-plane (in relation

to the steroid ring A), thus forcing the formed hydroxyl to shift under the ring plane and take the 3α-position [1, 3]. As a result of the reaction, the hydrogen and hydroxyl at the C3-carbon atom are oriented accordingly in relation to the product-steroid molecule plane, i.e., the hydrogen has β-orientation and the hydroxyl has α-orientation.

As concerns non-steroid substrates for 3α-HSD (prostaglandins, aromatic dihydroxy-compounds), hydroxyl *trans*-orientation in relation to the plane of the cycle containing these hydroxyls is a necessary condition, with 3α-HSD acting mainly as a dehydrogenase [3, 39].

X-Ray crystallographic analysis of the binary complex revealed that water also contributes to the reaction catalyzed by 3α-HSD. A water molecule is located close to the nicotinamide ring and forms hydrogen bonds between His117 and Tyr55 of the enzyme active site [3]. Moreover, the presence of a water molecule is likely to be really needed because the phenolic hydroxyl Tyr55 takes part in the reaction as a formal acid-base only under this condition (see below).

The binary complex binds with different steroids having a C3-ketogroup, but if a steroid cannot act as a substrate for 3α-HSD (e.g., a steroid molecule with a Δ^4-3-keto-structure), then competitive inhibition of the enzyme or even irreversible binding occur. This is especially true for testosterone and progesterone [28, 40]. The possible inhibition of 3α-HSD activity by testosterone and progesterone has, in our opinion, great physiological importance because it may be related to mechanisms of down regulation of 3α-HSD activity in rat brain (e.g., in pituitary) during puberty [41-44] or regulation of this activity in rat ovaries and brain after puberty [45].

The formation of the binary complex seems to be a necessary condition to facilitate and enhance substrate binding that results in a ternary enzyme–cofactor–substrate complex, though it is not always sufficient for completion of the reaction of 3α-HSD [4, 37] (see below). Formation of a formal or partial carbon ion of the substrate may occur in the transitional state of the ternary complex [3]. It seems that the C3-ketogroup is polarized by the Tyr55 phenol hydroxyl acting as a formal acid, and a hydrogen bond between the C3-ketogroup and His117 in the polypeptide chain of the enzyme is formed [28, 46].

Many details of the reaction catalyzed by 3α-HSD became clear when studying the crystal structure of the rat liver 3α-HSD–cofactor–testosterone complex [28]. The latter was chosen because it is structurally similar to DHT, i.e., a principle endogenous substrate for 3α-HSD, and forms a stable ternary complex available in crystalline form [28].

The active site of 3α-HSD consisting of four amino acids (Asp50, Tyr55, Lys84, and His117) is located deep in a hydrophobic cleft in which the substrate binding occurs; at the blind end of the cleft cofactor binding occurs [3, 47]. Study of several rat liver 3α-HSD variants obtained through directed mutagenesis demonstrated that Tyr55 plays an active role the active site. The phenol hydroxyl acts as an acid in reduction of 3-ketosteroid, and its effective pK_a is decreased (the pH optimum of the enzyme being 6.0, pK_a 5.6) due to formation of a hydrogen bond with Lys84 which in turn forms a bond with the carboxyl of Asp50 as a cation. In the dehydrogenase reaction (the enzyme pH optimum being 8.6, pK_a 8.9) Tyr55 acts as a base [1, 47], His117 contributes to the transition of the respective cofactor hydrogen onto the substrate carbonyl by the push–pull mechanism in the reduction reaction, and Lys84 takes part in the substrate hydrogen transport onto the cofactor in 3α-hydroxygroup oxidation [47].

The existence of an active site consisting of four amino acids is specific and identical for all enzymes from the large AKR superfamily including 3α-HSD [38, 47]. Replacement of any of these amino acids changes the activity of 3α-HSD considerably. For instance, a protein in which Tyr55 was replaced with Phe or Ser, and Lys84 was replaced by Met or Arg lost completely its enzymatic activity in relation to androsterone or 5α-androstane-3,17-dione [3]. On substitution of Asn or Glu for Asp50, the enzyme preserved only its dehydrogenase activity which was as little as 1/30 of that of native rat liver 3α-HSD activity. Substitution of Ala for His117 resulted in a 500-fold reduction in the protein activity as compared to the native enzyme [3]. The mentioned 3α-HSD variants preserved in full their ability to bind the cofactor (NADPH) and 3α-hydroxysteroid in parallel with a mild dehydrogenase activity but lost the ability to bind 3-keto-substrate and reductase activity [3], while after substitution of Phe or Ser for Tyr55 the enzyme retained its ability to form both the binary and ternary complexes [1]. This may have some relation to the fine organization of the 3α-HSD active site because the above-mentioned mutations fail to induce considerable disorder in the enzyme general spacial structure, affecting exclusively its active site.

Some nonspecific non-steroid anti-inflammatory drugs (NSAID) are effective non-competitive inhibitors of 3α-HSD. For instance, indomethacin at a concentration greater than 2.5 μM inhibited activity of both the microsomal and cytoplasmatic 3α-HSD forms in rat liver and skin [22, 35, 37], though human liver 3α-HSD isoforms were more readily inhibited by another NSAID class, i.e., 2-arylpropionic acid derivatives [21, 48] with one of them (AKR1C3) being inhibited most readily [49] (see also below).

It was demonstrated for rat liver 3α-HSD that indomethacin could form a complex both with the apoenzyme (having a low binding affinity) and the enzyme–NAD^+ binary complex (having high binding affinity) [37]. In the latter case indomethacin acted as a competitive inhibitor of substrate–steroid binding to yield a strong ternary complex similarly to testosterone [3]. All this suggests that tissular 3α-HSD may be a target for NSAID [3, 49].

3α-HSD activity is also inhibited effectively by barbiturates, pyrazole, and chloropromasin [11], which may be significant for regulation of the enzyme activvvity in brain using these drugs (see below). Some anti-lipidemic agents (of clofibrate type) may activate 3α-HSD in human liver and interfere with steroid metabolism [50].

The 3α-HSD inhibition–activation effects suggest that its isoenzymes can have different (often selective) binding sites for substrate, inhibitors, and activators including NSAID, whose effects are concentration-dependent [48]. However, the presence of a keto(hydroxy)group as an acceptor (donor) for the cofactor proton is a necessary condition for substrates, while the presence of a carbonyl group may be sufficient for a competitive inhibitor such as testosterone, progesterone, or NSAID (like indomethacin and 2-arylpropionates).

The above-described mechanism of 3α-HSD action including inhibition and activation suggests that its well-known polysubstrate activity may be related both to formation of a binary complex to react further with any ligand (substrate or inhibitor) approximately corresponding to the binding site structure on the enzyme molecule and with allosteric regulatory mechnisms (most likely for activation). Of most interest in this respect are prostaglandins as 3α-HSD substrates. It is likely that 3α-HSD activity towards 9-, 11-, and 15-hydroxyprostaglandins is a link between the action of these modulators and steroids on the cell, especially in inflammation [11, 35, 37, 48, 49, 51].

Most animals seem to have a single 3α-HSD form [52, 53] or AKR with 3α-HSD activity [34] in all tissues including liver. Some investigators believe that proteins with 3α-HSD activity from different animal tissues may be slightly different in structure due to post-translation modifications (e.g., rat liver, lung, kidney, testis [35]), though animal 3α-HSD heterogeneity is still discussed. Monkey liver may have several 3α-HSD isoforms [12], though some investigators disagree with this supposition [39] and a some of them consider the isolation of several 3α-HSD forms from animal liver an artefact [52].

A specific HSD from rabbit liver deserves special attention because it acts as 3α-, 3β-, 17β-, and 20α-HSD, demonstrates a number of unique features, and plays an important role in rabbit liver, though having a lower activity than other HSDs [34, 36].

There are four 3α-HSD isoforms in human liver, and these are referred to as AKR1C1-AKR1C4 [49]. All the four isoforms have different biochemical (mainly catalytic) properties depending upon amino acid sequence [39]. AKR1C1 seems to be specific for liver of humans only and possesses 20α-HSD activity [1]. This isoform demonstrates high activity to 3-keto/3α-hydroxygroups of various steroids including bile acids [39]. AKR1C2, also referred to as 3α-HSD type 3, binds bile acids with high affinity and is the main complex-former and bile acid carrier in the liver and probably in intestines [11]. The affinity of this isoform to bile acids is 1 or 2 orders of magnitude greater than that of rat liver 3α-HSD [5, 15]; therefore, the human liver AKR1C2 cannot act as 3α-HSD due to inhibition by bile acids [10, 11]. Some investigators believe that this human liver 3α-HSD isoform is not homogeneous, and the bile acid complex forming protein can be separated from the protein with 3α-HSD activity [15], while others consider these phenomena artefacts [14]. AKR1C2 demonstrates high activity to 3-keto/3α-hydroxyandrogens (but only in the absence of bile acids, e.g., in brain or prostate [9, 19]) and seems to be of much importance for human prostate [9], though it is expressed in other tissues too [19, 49, 50]. In animal liver the 3α-HSD acts as both a steroid metabolism enzyme and a complex former/bile acid carrier [15], and this seemingly accounts for different mechanisms of 3α-HSD inhibition by bile acids in human and animal livers. Of considerable interest is the isoform AKR1C3, sometimes also referred to as 3α-HSD type 2. Besides liver, this 3α-HSD isoform is found in other human tissues (kidney, lung, brain, heart, spleen, adrenals, small intestine, placenta, prostate, testicles) and demonstrates low affinity and activity to DHT, zero activity to bile acids, and high specific activity to prostaglandins and 3-keto/3α-hydroxy-5β-androgens and possesses 17β-HSD activity [11, 49]. AKR1C4 (or 3α-HSD type 1) is most specific for human liver and demonstrates the greatest (as compared to other human liver 3α-HSD isoforms) affinity to DHT and the highest activity to 3-keto/3α-hydroxysteroids [11]. It should be emphasized that the human 3α-HSD isoforms are differently inhibited by endogenous and exogenous factors [49, 50] (see also above).

The human 3α-HSD isoforms demonstrate a great variability of properties though having 83-98% identical amino acid sequences [49]. These are genetically different proteins, though post-translational modification of the isoenzymes may take place and also have a relation to the microheterogeneity of these proteins from different tissues [53].

So, notwithstanding some contradictions concerning 3α-HSD forms between different authors, certain conclusions may be made about the significance of the enzyme in human and animal tissues. All human 3α-HSD isoforms act as enzymes in redox reactions of steroid 3-keto/3α-hydroxygroups, however each isoenzyme not only demonstrates different activity in these reactions but also has different substrate specificity in different tissues to endogenous and exogenous compounds including steroids. Besides, there are at least two isoforms in many human tissues (except for liver), one of which demonstrating high activity to 3-keto/3α-hydroxysteroids and the other demonstrating a lower activity to these compounds in parallel with higher substrate specificity. In our opinion this observation is of great physiological significance because it follows that steroid metabolism in various tissues may be regulated both by steroids

themselves and by other endogenous compounds to which 3α-HSD isoforms demonstrate activity. The presence of only a single enzyme form with 3α-HSD activity in most animal tissues (also acting as complex-former and bile acid carrier in liver and intestine) suggests that regulatory mechanisms of 3α-HSD activity in animal tissues may differ from those in human tissues [34, 36, 39].

THE ROLE OF 3α-HSD IN ANIMALS AND HUMANS

Notwithstanding the high prevalence and the vast study of 3α-HSD in animal and human tissues, the physiological significance of the enzyme is still disputed, especially as concerns its activity regulation, polysubstrate specificity, and role in mechnism of steroid action. Discussion of the role of 3α-HSD in all animal and human tissues is hardly possible here, and this review therefore focuses only on the most important issues related to the action of steroids produced in some tissues due to the contribution of the enzyme.

In rat gonads 3α-HSD plays a significant role in biosynthesis of androgens and likely progestins by the 5α-pathway [54] during puberty of both rat genders [17, 24, 45, 55, 56]. The reductase activity of the enzyme and its expression in testes reaches a maximum in prepubertal rats and decreases with age [17]. Young mouse testes synthesize mainly 3α-hydroxyandrogens too [57]. In rat ovaries 3α-HSD activity is rather high after puberty too, though the spectrum of steroids in whose biosynthesis 3α-HSD takes part (3α-D in prepubertal and 5α-pregnane-3α,20α-diol in pubertal animals) may be different [56]. Besides, after the rat vagina is opened ovarian 3α-HSD activity becomes dependent upon the sex cycle [55]. There are species-specific differences in 3α-HSD activity from ovaries of some animals, e.g., there is practically zero synthesis of 3α-hydroxysteroids in guinea pigs aged 20-40 days, hamsters synthesize mainly 5α-pregnane-3α,17α-dihydroxy-20-one (3α,17α-di-OH-Pr) up to day 20 of life, while rats aged 30 days synthesize equal amounts of 3α-D and 3α,17α-di-OH-Pr [58].

The role of 3α-HSD in human and animal liver is not quite clear, though the enzyme activity was first discovered in liver and the enzyme itself is thought to be absolutely indispensable for normal liver function, e.g., in directed transport and biosynthesis of bile acids [3]. One of the functions of 3α-HSD in liver is likely to contribute to steroid catabolism resulting in formation of 3α-hydroxysteroids, that in turn can be converted into glucuronides [59]. The latter is the easiest way to render steroids soluble because UDP-glucuronyl transferases act very effectively on 3α-hydroxysteroids [59]. However, based on the polysubstrate activity of 3α-HSD, especially in humans (see above), the supposition can be made that the enzyme also makes a significant contribution to detoxication and/or metabolic activation of xenobiotics, in particular polycyclic aromatic carbohydrates, though the K_m for xenobiotics is 2-3 orders of magnitude greater than that for steroids [3, 11, 60].

Besides intensive metabolism of androgens [61], lung 3α-HSD performs the function of xenobiotic (carcinogen) detoxication and (probably) interferes in the development of asthma (through prostaglandin metabolism) [50]. In small intestine (another site with high 3α-HSD activity [62]) the enzyme participates in the transport of bile acids and xenobiotics [10]. In kidneys 3α-HSD contributes both to steroid [13] and prostaglandin [50] catabolism. The 3α-HSD activity in sheep red cells [63] and human platelets [64] may be related to steroid catabolism, though 3α-HSD products may also be responsible for specific functions in these cells.

Brain is a site in which 3α-HSD plays a significant and probably second to liver most important role. Already in the early forties of this century H. Selye demonstrated that 3α-hydroxysteroids can induce anxiety and produce anticonvulsive and analgesic effects in animals [65]. As shown recently, these steroids may interfere in alimentary processes through central mechanisms [66]. It was as late as in the eighties that a molecular mechanism was proposed to explain the action in brain of 3α-hydroxysteroids or neurosteroids (another term for these metabolites produced in brain). This mechanism bore no relation to the classical (receptor-mediated) mechanism of steroid action, but rather is an illustrative example of non-genomic mechanisms of action (see below). The neurosteroids first of all include 3α-hydroxy-5α-reduced glucocorticoids and progestins (tetrahydrocorticosterone, tetrahydroprogesterone, pregnanolone) that can be actively produced by the body under stress [19]. 3α-HSD is thought to play a key role in neurosteroid production and action in brain since it is the principal enzyme in their synthesis in the central nervous system, though 5α-R also makes a considerable contribution, in particular in the pituitary and hypothalamus [41-43, 67].

The neurosteroids must play an important role in the peripheral nervous system too. For instance, pregnanolone may increase gene expression of some myelin proteins whose synthesis is reduced with age in rats: pregnanolone administration to aging animals resulted in activation of the protein genes, gene expression increased significantly in the presence of pregnanolone in Schwann cell culture from neonatal animals [68].

The neurosteroids are agonist ligands or allosteric effectors for the $GABA_A$-receptor complex [3, 19]. When binding to the latter the neurosteroids induce ion channel opening and neuron hyperpolarization [69, 70]. Calcium channel conduction in neurons may be finely regulated by 3α-hydroxy-5α-androgens [71]. Note that these effects take place no matter what configuration (5α- or 5β-reduction) the neurosteroid ring A has or whether the steroid acts in the free state or (frequently) as sulfate [70,

72]. The action of neurosteroids is due to specific binding sites just for these ligands rather than to the effect on their lipid membrane [71, 73].

The interaction of 3α-hydroxysteroid with $GABA_A$-receptor complex is related to the neuronal response to stimulation and defense from overstimulation needed to maintain central nervous system homeostasis [19, 74].

Some investigators has demonstrated that 3α-hydroxysteroids bind to $GABA_A$-receptors near binding sites for barbiturates, benzodiazepines, and ethanol [19, 75, 76].

The $GABA_A$-receptor subunit structure undergoes changes during ontogenesis [51] and during pregnancy and parturition in rats [75-78], and $GABA_A$-receptor sensitivity to neurosteroids may therefore change too. It follows then that $GABA_A$-receptor activity may be related both to their structural changes and to changes in brain 3α-HSD activity (expression) and/or composition of gonadal (adrenal) steroids during pregnancy or in ontogenesis [77-79].

3α-HSD demonstrates the highest activity in olfactory bulbs [19] in which high concentrations of $GABA_A$-receptors are also found [69].

Brain 3α-HSD expression and activity regulation is directly related not only to the protective function of 3α-hydroxyneurosteroids [19, 67, 80] but also to sexual behavior, especially as concerns rat and mouse females (through smell reception and/or LHRH regulation) [20, 49, 81] and mobility [76]. This enzyme is of importance for sex differentiation of the brain [60, 63] and pituitary gonadotropic function in rats [82-85], though there are different opinions on this matter [67].

The modulating action of 3α-hydroxysteroids on $GABA_A$-receptors seems to underlie some mental abnormalities such as depression, anxiety, premenstrual syndrome, and others. They may be due to a disorder of steroid (especially neurosteroid) metabolism both in the brain [1, 67, 86, 87] and source sites of these steroids, i.e., adrenal for the first two and ovaries for the latter [9, 88, 89]. These findings suggest that 3α-HSD is of great importance for the action of some steroids in the animal and human brain [20, 67].

The activity of the enzyme is regulated both by endocrine and intracrine mechanisms with not only steroids being responsible for the regulatory function. Changes in brain 3α-HSD in the hypothalamus–pituitary–adrenals under stress [80], in the hypothalamus–pituitary–gonads in adult rat females during sexual behavior [20, 45, 81] or sex-specific differentiation of brain [67] are typical examples of the endocrine regulation of the enzyme. Rat liver glucocorticoids may regulate 3α-HSD activity at the gene transcription level [90], while progestin, androgen, estrogen, prostaglandin, arachidonic and bile acid regulation is associated with the active site of the enzyme [4, 5, 50]. Prostaglandins and retinoic acid probably regulate 3α-HSD activity by an allosteric mechanism [4, 68].

Figure 1 presents a general picture of the role of 3α-HSDs in some human and animal tissues that are of the most importance from the point of view of the value of the enzyme for these tissues. As seen, 3α-HSDs both plays a common role for all tissues in which it is found and performs specific functions characteristic for some tissues.

Numerous findings on the physiological significance of 3α-HSD suggest that this enzyme plays a very important (most likely exclusive in brain and peripheral nervous system) role in all tissues in which it is found irrespective of whether DHT or other 5α-reduced steroids are produced in the tissue [67].

We believe that the physiological significance of 3α-HSDs in different tissues, schematically presented in Fig. 1, is due to the principal role of the enzyme in the mechanism of action of steroids (at least in some tissues) related to production of 3α-hydroxysteroids and/or maintenance in these cells of dynamic balance between 3α-hydroxy- and 3-ketosteroids. Assuming this, the following pattern may be suggested for the participation of 3α-HSD in the mechanisms of action at the cell level of three types of steroids (i.e., androgens, progestins, and glucocorticosteroids) that are known to possess features of both genomic (through receptor mechanisms) and non-genomic (not related to classical receptors) regulators of cell processes [2, 91-93].

The main Δ^4-3-ketosteroids penetrate into cells from the blood. Progesterone and glucocorticosteroids (corticosterone and cortisol) act directly as genomic regulators through specific cytoplasmic receptors (Fig. 2). Testosterone may also contribute to the regulation through interaction with cytoplasmatic receptors, while DHT (produced from testosterone under the effect of 5α-R) is the most important effector hormone for androgens [66, 93, 94]. Most Δ^4-3-ketosteroids (4-androstene-3,17-dione and testosterone, probably completely) change into 5α-3-ketosteroids (Fig. 2: 5α-ANDR, 5α-DHPR, and 5α-COR) under the action of 5α-R. At this stage a part of the DHT produced acts as a regulator at the gene level. Some investigators believe that 5α-DHPR may also act through or modulate the activity of progesterone receptors, e.g., in the brain in rat female sexual behavior [81, 95]. Most 5α-reduced steroids (and probably a part of 5β-3-ketosteroids penetrating into cells from the blood) are converted into 3α-hydroxy-5α(β)-steroids under the activity of 3α-HSD and may function as non-genomic regulators (Fig. 2). Known reactions with 5α-R are irreversible, while 3α-HSD catalyses reversible reactions. We think the latter peculiarity to be of extreme importance because it allows 3α-HSD to regulate cell concentrations of both 3α-hydroxy- and 3-ketosteroids [95], e.g., to maintain the DHT cell concentration needed to saturate androgen receptors [22] in tissues without or with low 5α-R activity [24].

Most 3α-hydroxysteroids are likely to be converted under the effect of UDP-glucuronyl transferases into glu-

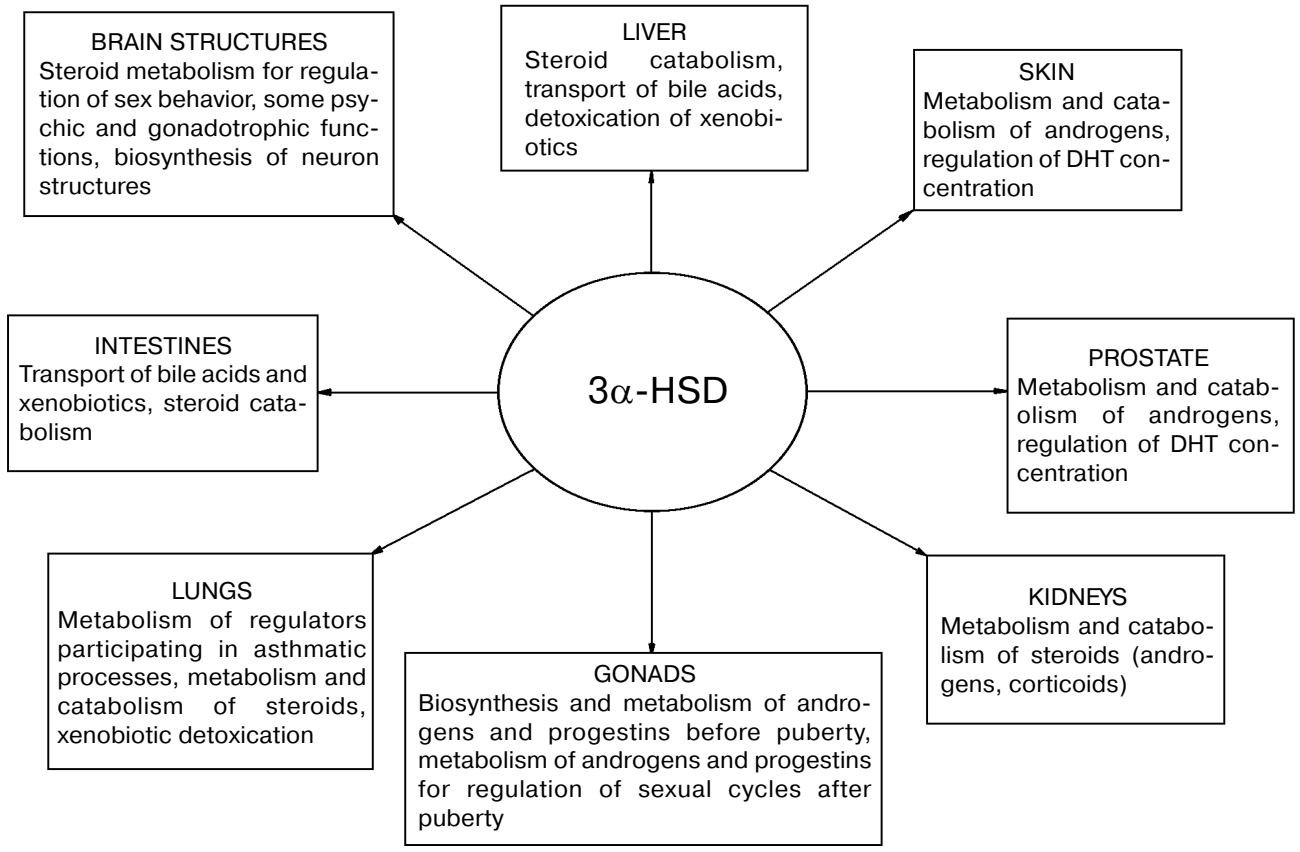

Fig. 1. Basic actions 3α-HSD in human and animal tissues.

curonides that are removed from the cell. The glucuronides can change into free steroids in some cells, which may also be related to regulation of cellular contents of 3α-hydroxysteroids [59].

It may also happen that 3α-HSDs contribute to the regulation of orphan receptor functioning, in particular of CAR-β receptors by interfering with the production of their specific endogenous ligands, i.e., androstanol (5α-androstane-3α-ol) and androstenol (5α-androstane-16-ene-3α-ol) [96, 97]. The 3α-HSD may act as a switch of the orphan receptor functioning since 5α-reduced 3α-hydroxysteroids are extremely effective endogenous inhibitors of the activity of these receptors as demonstrated, for instance, for CAR-β receptors [96].

So, the intracellular production of 3α-hydroxysteroids catalyzed by 3α-HSD is not only inactivation of 3-ketosteroids (or a switch for DHT functioning [1]) but also (or even rather) a process for the production of non-genomic regulators with different activities in different cells. We believe that it is 3α-HSD that plays in these processes the role of a switch or regulator of 3-ketosteroid genomic action (through receptor mechanisms) to (with 5α-R contribution) non-genomic action, i.e., direct cytoplasmic and membrane effects [2, 91, 93, 95, 98]. This supposition seems to be especially true for brain and prostate.

It is now established that 3α-HSD is both an important enzyme of steroid metabolism and a necessary regulator of the mechanism of steroid action, though there is much still to be elucidated in this matter. Answers to many questions may be found during further study of the non-genomic action of various 3α-hydroxysteroid groups [2, 91] and exact mechanisms of their action in different cells.

It should be emphasized that it is still unclear what underlies 3α-HSD specificity to endogenous substrates (androgens, progestins, and glucocorticosteroids) and how they interact in their common effects on the activity of the enzyme in tissues. These problems are related to the role of 3α-HSD polysubstrate activity in production and mechanisms of action of both 3α-hydroxysteroids and other biologically active compounds that may act as substrates or catalysis products of the enzyme, as well as to their interrelationship with steroid action [4, 11, 51]. 3α-HSD activity may not always be indicative of the role of respective steroids in a particular tissue but rather be related to the role of other substrates of the enzyme. Study of details of the polysubstrate activity of 3α-HSD

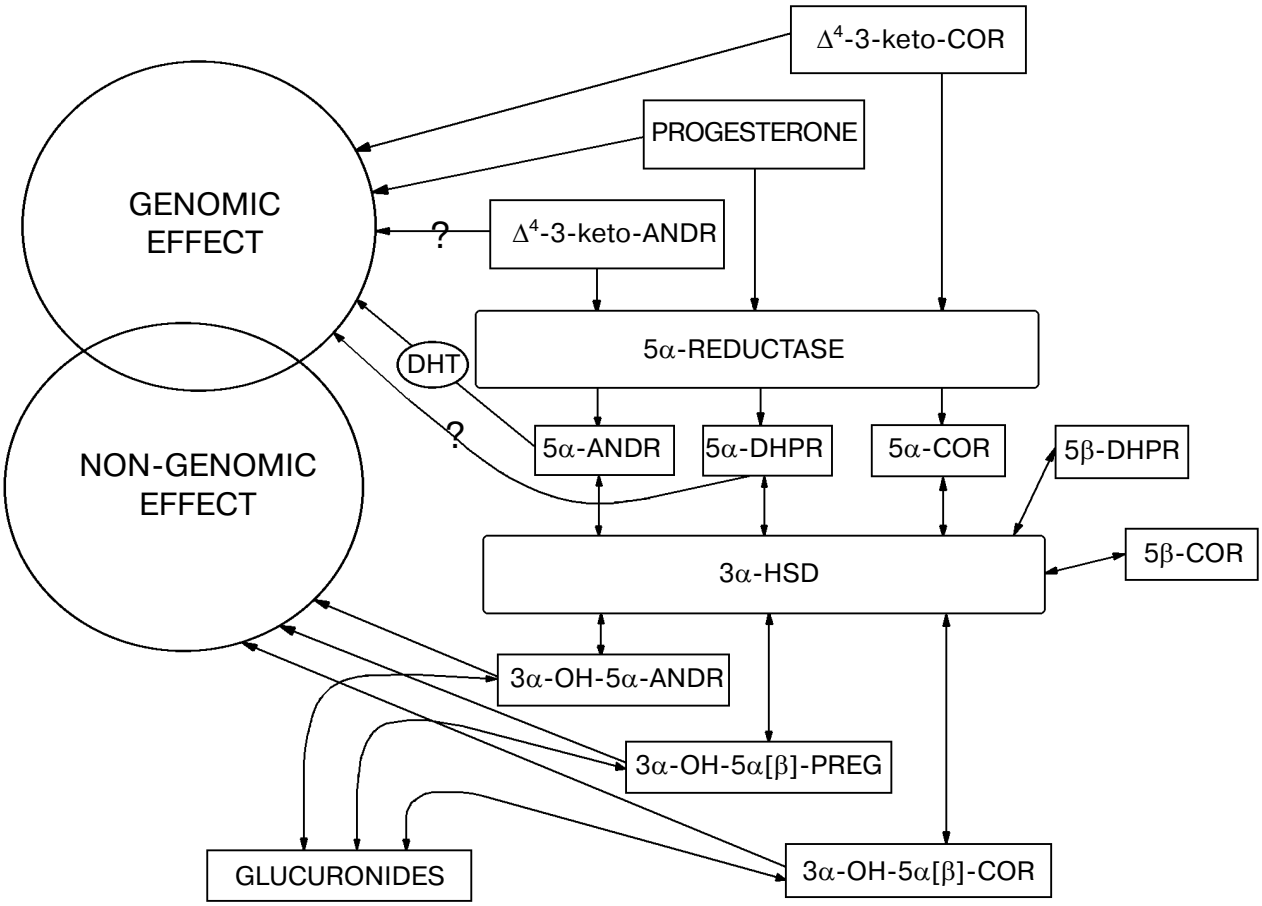

Fig. 2. Pattern of the contribution of 3α-HSD to steroid genomic and non-genomic effects in target cells. Conventional designations: Δ^4-3-keto-HCOR, corticosterone (4-pregnene-11β,21-dihydroxy-3,20-dione) or cortisole (4-pregnene-11β,17α,21-trihydroxy-3,20-dione); Δ^4-3-keto-ANDR, 4-androstene-3,17-dione and 4-androstene-17β-hydroxy-3-one (T); 5α-ANDR, 5α-androstane-3,17-dione and 5α-dihydrotestosterone (DHT); 5α-DHPR, 5α-dihydroprogesterone (5α-pregnane-3,20-dione); 5α-HCOR, *allo*-dihydrocorticosterone (5α-pregnane-11β,21-dihydroxy-3,20-dione) or *allo*-dihydrocortisole (5α-pregnane-11β,17α,21-trihydroxy-3,20-dione); 5β-DHPR, 5β-pregnane-3,20-dione; 5β-HCOR, dihydrocorticosterone (5β-pregnane-11β,21-dihydroxy-3,20-dione) or dihydrocortisole (5β-pregnane-11β,17α,21-trihydroxy-3,20-dione); 3α-OH-5α-ANDR, androsterone and 3α-D; 3α-OH-5α[β]-PREG, 5α(β)-pregnane-3α-ol-20-one; 3α-OH-5α[β]-COR, 5α(β)-pregnane-3α,11β,21-trihydroxy-20-one or 5α(β)-pregnane-3α,11β,17α,21-tetrahydroxy-20-one. "?", steroid action has not been exactly determined.

in animals and humans is important from both the theoretical and practical standpoints because this polyfunctional enzyme may be a target for many compounds (endogenous and xenobiotic), which opens wide prospects for specific regulation of its activity in various tissues [11].

Such a study will elucidate roles not only of 3α-HSDs but also of steroid reactions with respect to the C3-carbon and other possible substrates for 3α-HSD in regulatory processes in cells of different tissues, in particular brain, liver, prostate, and gonads [24, 28].

It is also unclear which of the activities (reductase or dehydrogenase) is preponderant in particular tissues though it is most likely that an equilibrium (buffer) system 3-keto-5α(β)-reduced steroid ↔ 3α-hydroxysteroid with 3α-HSD [22-24, 99] is normally effective or the reductase activity predominates [3, 5, 6, 11, 19, 100]. The reductase or dehydrogenase activity may be manifestations of activities of 3α-HSD isoforms [100]. Or the activity of every 3α-HSD isoform may depend upon the conditions of the production of the binary complex (as a reaction limiting stage) before it combines with a substrate [87].

Another important problem is the significance of 3α-HSD microsomal and soluble (cytoplasmic) forms and their isoforms that certainly play important roles in cells of steroid producing tissues and in liver, particularly in man [1, 3, 5, 6, 19]. Of much importance here are both 3α-HSD activity measurement by adequate and unified

methodology and establishing correlation between the activity and expression of the enzyme, because the enzyme isoforms may be produced as a result of post-translation processing of a proform, as demonstrated in several studies [19, 39].

The achievements and potential of gene engineering open vast prospects for comprehensive study of the role of 3α-HSD in animal and human tissues. Gene engineering methods may help to design 3α-HSD modifications with different activities depending upon changes in substrate specificity and/or allosteric regulation by some compounds, as demonstrated recently [101].

In conclusion, although 3α-HSD is one of the best studied enzymes from the AKR superfamily, it is no overestimation to say that equivocal findings concerning this enzyme and reactions it catalyzes make it a most enigmatic participant of steroid production and therefore open as a broad field for study.

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