DEHYDROEPIANDROSTERONE METABOLITES AND THEIR INTERACTIONS IN HUMANS

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SUMMARY

Dehydroepiandrosterone (DHEA) is 7α-hydroxylated by the cytochrome P4507B1 in the liver, skin and brain, which are targets for glucocorticoids. 7α-Hydroxy-DHEA produced anti-glucocorticoid effects *in vivo* but the interference between the glucocorticoid hormone binding with its receptor could not be determined. In the organs mentioned above, circulating inactive cortisone is reduced to active cortisol by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). 7α-Hydroxy-DHEA is also a substrate for this enzyme. Studies of 11β-HSD1 action on 7α-hydroxy-DHEA show the reversible production of 7β-hydroxy-DHEA through an intermediary 7-oxo-DHEA. Both the production of 7α-hydroxysteroids and their interference with the activation of cortisone into cortisol are basic to the concept of native anti-glucocorticoids. The cytochrome P4507B1 responsible for 7α-hydroxy-DHEA production and 11β-HSD1 are key enzymes for the modulation of glucocorticoid action in humans. This is a promising new area for research.

KEY WORDS

steroid, 7α-hydroxylation, metabolism, hydroxysteroid dehydrogenase, glucocorticoid

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INTRODUCTION

Dehydroepiandrosterone (DHEA) in humans is produced by the adrenals and gonads. Prior to puberty, and at the moment of adrenarche, the adrenals become the main DHEA producer. DHEA is then produced in the gonads as a precursor for steroid sex hormones (testosterone and estradiol) /1/. The human ability to convert DHEA into its sulfate derivative (DHEAS) is less extensive or does not occur in other non-primate species /1,2/. Circulating DHEAS levels in humans are three orders of magnitude higher that those of DHEA /2/, but barely detectable in rats and mice. Nevertheless, free DHEA plasma levels are in the nM range in humans, rats and mice /1,2/. In both sexes, DHEA human plasma levels decrease with aging /3/, which led to its extensive use as a 'youth hormone' /4/, despite multiple disputes regarding its targets and assessment of its real effects. Finally, after work was carried out *in vivo* with rats and mice, DHEA was described as a native anti-glucocorticoid /5/ that promotes the immune response /6-8/ and as a neuroprotective agent /9/. These results could neither be confirmed nor explained when *in vitro* approaches were used for investigation of DHEA's mode of action, and there was speculation that DHEA metabolites could be responsible for the effects obtained *in vivo* /5/. The classical metabolism of DHEA has been extensively studied and reviewed with the aim of showing its transformation into sex steroid hormone precursors and metabolites. This led to the concept of 'intracrinology' in which many tissues and organs were found responsible for the conversion of DHEA into androgens and estrogens /10,11/. Nevertheless, few investigators provided evidence for the hydroxylation of DHEA. Hydroxylated derivatives of DHEA are scarcely available and their occurrence was mainly sought in the cytochrome P450-rich liver. As the liver is responsible for xenobiotic (including steroids) elimination through Phase I (cytochrome P450-mediated hydroxylation) and Phase II (conjugation) processes, it was merely concluded that hydroxylated derivatives were solely aimed at depleting the organism of excessive steroid production.

7α-Hydroxy-DHEA is one of the major DHEA metabolites produced in the liver of mice /12,13/, and it became evident that 7α-hydroxy-DHEA was also produced in several other tissues and organs, including the brain, lymph glands and skin /8/. Comparison of DHEA

and 7α-hydroxy-DHEA effects on the immune response in mice showed that small doses of 7α-hydroxy-DHEA promoted the immune response much better than large doses of DHEA /8/. Androgenic steroids are derived from DHEA after puberty and include epiandrosterone (EpiA) which shares with DHEA a closely similar structure allowing its 7α-hydroxylation /14/. The 7-hydroxylated derivatives of EpiA are of interest because of their reported neuroprotective effects /15,16/. Thus, DHEA metabolism is the key to understanding several physiological events.

To date, most of the data are derived from animal studies and can not be readily applied to humans. The need for normal tissues from human beings could only be satisfied in limited amounts with commercial preparations from pools, and variations within them excluded extensive enzyme kinetic studies. Therefore, the cDNAs of selected human steroid-metabolizing enzymes were used for yeast transformation and expression of the desired functional proteins /17-19/. The steroid substrates were radiolabeled in order to follow and measure their transformation products /20/. These methods were applied to the examination of DHEA, EpiA and related 7-hydroxylated derivatives, and to the kinetic studies of their transformation by the two human steroid-metabolizing enzymes.

7α-HYDROXYLATION BY CYTOCHROME P4507B1 (CYP7B1)

In the human liver several cytochromes P450 were shown to carry out the 7α-hydroxylation of DHEA. One of the most abundant cytochromes in the liver is the CYP3A form, and CYP3A4 and CYP3A5 were reported to carry out 7α-hydroxylation of DHEA /21,22/. Another cytochrome P450 responsible for the 7α-hydroxylation of DHEA has been identified in the livers of rats, mice and humans, and termed 7B1 (CYP7B1) /23-25/. In humans this cytochrome and the 7α-hydroxylation of DHEA occur in the liver /25/, brain /26-28/, skin /29/ and tonsils /30/. The steroid substrates for CYP7B1 are numerous and bear in common the 3β-hydroxy group and a ketone or hydroxyl group that may occur at any position from 17 to 27 (Fig. 1). It has been reported that the CYP7B1-catalyzed 7α-hydroxylation of oxysterols in the liver was a first step for bile acid synthesis and constituted an alternative pathway to that of cholesterol 7α-hydroxylation by CYP7A1 /31/ (Fig. 2). Studies of human

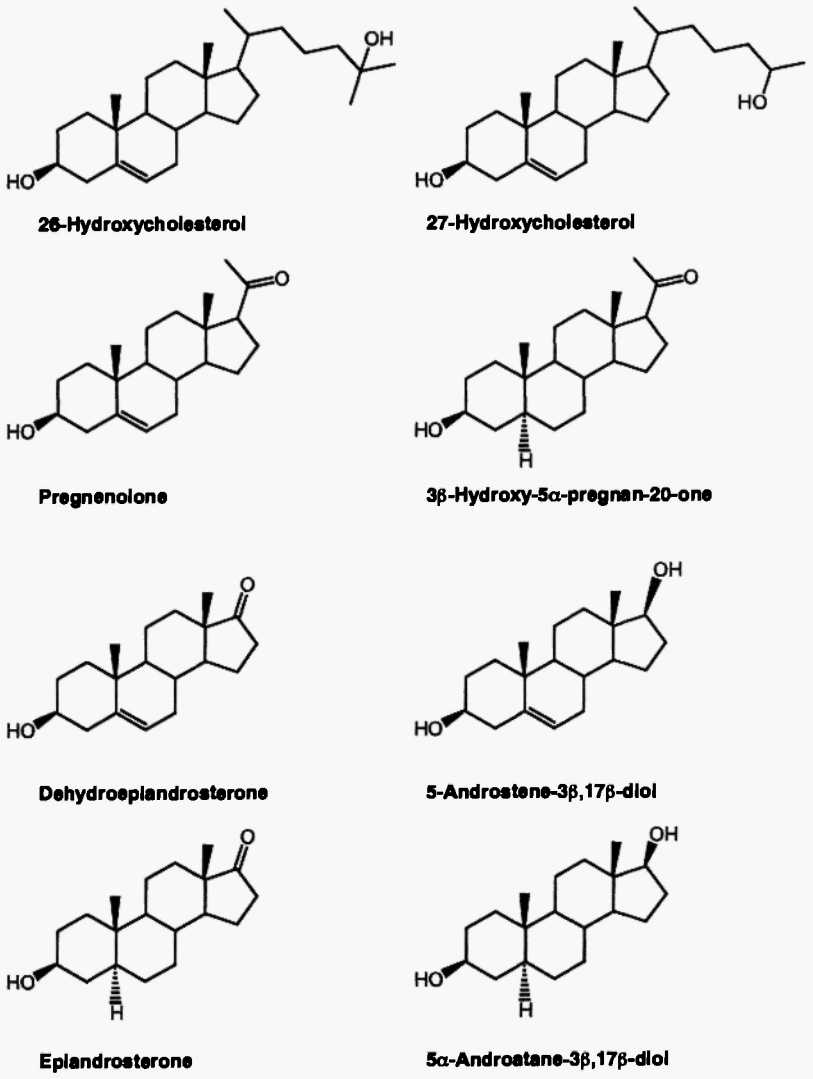

Fig. 1: Steroid substrates for the 7α-hydroxylating cytochrome P4507B1.

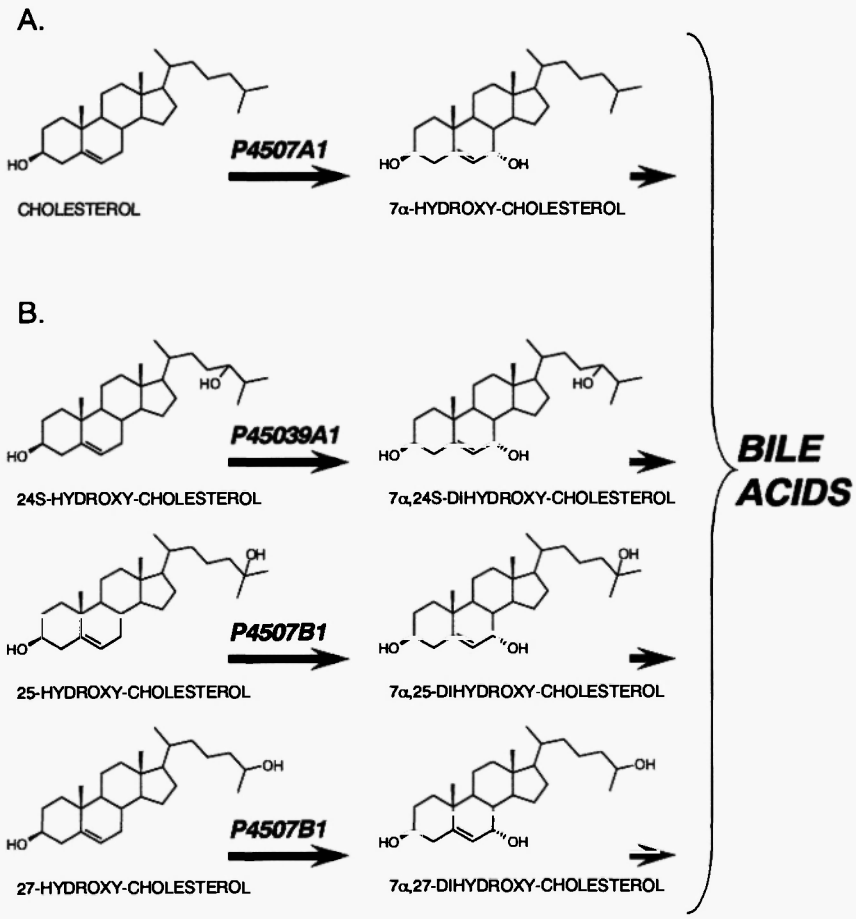

Fig. 2: Pathways A and B available for bile acid production in the liver.

CYP7B1 catalytic activities were achieved after the human protein was expressed in yeast microsomes /14/. K_M and K_{cat} were measured after the incubation of [4-^{14}C]-labeled steroid substrates, including pregnenolone, DHEA, EpiA, 5α-androstane-3β,17β-diol and estradiol. The preferred substrate was DHEA, as indicated by its highest K_{cat}/K_M ratio (Table 1). Surprisingly, the major 7α-hydroxylation of these

TABLE 1

Kinetic parameters obtained for human P4507B1 with several steroid substrates /27/

Steroid substrate	7α-Hydroxylation			7β-Hydroxylation		
	K_M [a]	K_{cat} [b]	K_{cat}/K_M	K_M [a]	K_{cat} [b]	K_{cat}/K_M
DHEA	1.90 ± 0.05	3.14 ± 0.10	1.653	2.38 ± 0.27	0.088 ± 0.009	0.037
EpiA	1.05 ± 0.12	0.167 ±0.006	0.159	1.05 ± 0.12	0.073 ± 0.002	0.069
5α-Androstane-3β,17β-diol	0.80 ± 0.04	0.54 ± 0.03	0.675	0.80 ± 0.04	0.073 ± 0.004	0.091
Pregnenolone	1.45 ± 0.03	0.22 ± 0.01	0.152	ND	ND	
Estradiol	1.35 ± 0.30	0.007 ± 0.001	0.005	ND	ND	

[a] μM ± SEM; [b] pmol.min^{-1}.pmol CYP7B1^{-1} ± SEM; ND = not detected.

substrates was accompanied with a minor 7β-hydroxylation that did not occur for pregnenolone and estradiol. Because of the identical kinetic parameters found for both 7α- and 7β-hydroxylations (Table 1), because of the more stable thermodynamic state of the 7β-epimer, and because of the precedent found with the enzyme from *Fusarium moniliforme* /32/, a side reaction was suspected to be carried out by CYP7B1. Other research used both DHEA and 27-hydroxycholesterol substrates with transfected human kidney 293/T cells, and it was found that the 7α-hydroxylating activity for DHEA is 100 times higher than for 27-hydroxycholesterol /25/.

Both EpiA and 5α-androstane-3β,17β-diol are some of the androgen metabolites formed after puberty, and their irreversible 7α-hydroxylation is of interest because it may provide inactivation of androgen potencies and generation of other putatively potent metabolites. Thus, neuroprotective effects were reported for both 7α- and 7β-hydroxy-EpiA /15,16/, and their prospective pharmacological use as neuroprotective agents is under investigation.

7β-HYDROXYLATED METABOLITES

When the steroid content of human plasma was examined, both 7α- and 7β-hydroxy-DHEA were detected in almost equivalent amounts /33-35/. Such quantities of 7β-hydroxy-DHEA could not be explained with the low amounts produced by CYP7B1 /14/. Previous investigations found CYP1A1 responsible for pregnenolone 7β-hydroxylation, but DHEA 7β-hydroxylation could not be found among cytochromes 1A1, 1A2, 3A4, 3A5, 2C8, 2C9, 2C18, 2C19, 2D6 and 2E1 /36/. However, the 7β-hydroxylation of DHEA by CYP3A4 has recently been documented /22,37/. Another study with CYP7B1 knock-out mice showed that neither 7α-hydroxy-DHEA nor 7β-hydroxy-DHEA were produced /38,39/. This implied that the 7α-hydroxylation of DHEA by CYP7B1 was a prerequisite for 7β-hydroxy-DHEA production. Since the presence of a specific CYP for the 7β-hydroxylation of DHEA may be discounted, the 7β-hydroxy-DHEA production may only involve either an isomerase-driven or an oxidoreductase catalyzed process. The presence of a specific isomerase transforming 7α-hydroxy-DHEA into 7β-hydroxy-DHEA can be excluded because the percutaneous administration of 7-oxo-DHEA in humans led to an increase of both circulating 7α- and 7β-hydroxy-

DHEA /40,26/. Previous research in liver preparations from animals and humans demonstrated that the 11β-hydroxysteroid dehydrogenase type 1 (11β-HDS1) was responsible for the conversion of 7α-hydroxy-DHEA into 7-oxo-DHEA /41,42/. When the NADPH-dependent reduction of 7-oxo-DHEA by human liver microsomes was compared with 7β-hydroxy-DHEA production, a 1:2 ratio was obtained /41/. The whole reduction was significantly inhibited by the non-selective 11β-HSD1 inhibitor, carbenoxolone, while dehydrocorticosterone (one substrate for 11β-HSD1) inhibited only 7α-hydroxy-DHEA production. These findings contributed to the conclusion that an unidentified reductase in the liver caused the NADPH-dependent 7-oxo-DHEA reduction into 7β-hydroxy-DHEA. More recently, and with use of the yeast-expressed human 11β-HSD1, we could prove that this enzyme was solely responsible for the interconversion of 7α-hydroxy-DHEA and 7β-hydroxy-DHEA via the 7-oxo-DHEA intermediate /43/. Whether the same process applies to the interconversion of 7α-hydroxy-EpiA and 7β-hydroxy-EpiA is presently under investigation. This finding is important because it proves that both 7α-hydroxy-DHEA and 7β-hydroxy-DHEA levels result from CYP7B1 production and from the presence of 11β-HSD1.

INTESTINAL METABOLISM OF DHEA AND EpiA, AND THEIR RESPECTIVE 7-HYDROXYLATED METABOLITES

Neuroprotection exerted by the production of 7α-hydroxy-DHEA was first suggested after work with rat hippocampal cell cultures and comparison with aromatization products /44/. More recently, a test for that hypothesis was developed on a model of hypoxia-induced neurodegeneration in organotypic rat hippocampal slice cultures /16/ and on an *in vivo* model of cerebral ischemia obtained in rats by four-vessel occlusion /15/ in which several 7-hydroxylated steroids were tested for their neuroprotective action. It was clearly demonstrated that DHEA, EpiA, 7-oxo-EpiA, 5α-androstane-3β,7β,17β-triol, and estradiol had no neuroprotective effects, while 7α-hydroxy-DHEA, 7α-hydroxy-EpiA and 7β-hydroxy-EpiA prevented neuronal damage 24 h after the onset of hypoxia /15/. Among the steroids tested, 7β-hydroxy-EpiA (0.03 mg/kg injected in the femoral vein) was the most efficient for neuronal recovery from hypoxia, and this suggested that native 7β-hydroxysteroids may exert a neuroprotective function /15/.

Possible pharmacological use *per os* of any of the neurosteroids described (i.e. DHEA, 7α-hydroxy-DHEA, 7β-hydroxy-DHEA, EpiA, 7α-hydroxy-EpiA, 7β-hydroxy-EpiA) implies that metabolism in the intestine will subsequently determine their availability to the liver and brain. Therefore, we studied the yields of neurosteroid transformations in microsomes derived from human intestine and identified the metabolites produced. To achieve this we produced the [4-^{14}C]-labeled steroid substrates /20/ and incubated them under different cofactor supplementations with microsomes of human intestine. The metabolites produced were identified by gas chromatography/mass spectrometry /45/. The sole metabolites resulting from either DHEA or EpiA incubations were their respective 17β-reduced derivatives. The largest yields were obtained after NADH supplementation, while NADPH supplementation resulted in three times less (Fig. 3). This implied that no CYP7B1 and both NADH- and NADPH-dependent 17β-hydroxysteroid oxidoreductases were present in the intestinal microsomes. The same finding applied to 7α-hydroxy-DHEA and 7β-hydroxy-DHEA metabolism where 17β-reduction resulted in the respective triol production (Fig. 3). In contrast, incubation with either 7α-hydroxy-EpiA or 7β-hydroxy-EpiA provided evidence for interconversion of the two steroids in addition to their 17β-reduction. No trace of a 7-oxo derivative was detected, and one may question the involvement of an oxidoreductive process in this interconversion /45/ (Fig. 3).

Other work carried out *in vivo* on rats was aimed at the antioxidant effects of DHEA and 7α-hydroxy-DHEA in the colon, intestine and liver /46-48/. Pelissier *et al.* administered the steroids *i.p.* (50 mg/kg b.wt.) to healthy male Wistar rats for two or seven days and observed protective effects, particularly in the colon where they increased goblet cell proliferation and mucus production. It was apparent that the effects of 7α-hydroxy-DHEA were exerted earlier than those of DHEA /48/. This led to the conclusion that DHEA was the precursor for the active 7α-hydroxy-DHEA metabolite. Administration of either steroid was also efficient for protection against sodium dextran sulfate-induced colitis in rats, with restoration of the diseased colon to normal histological aspect and function /49/.

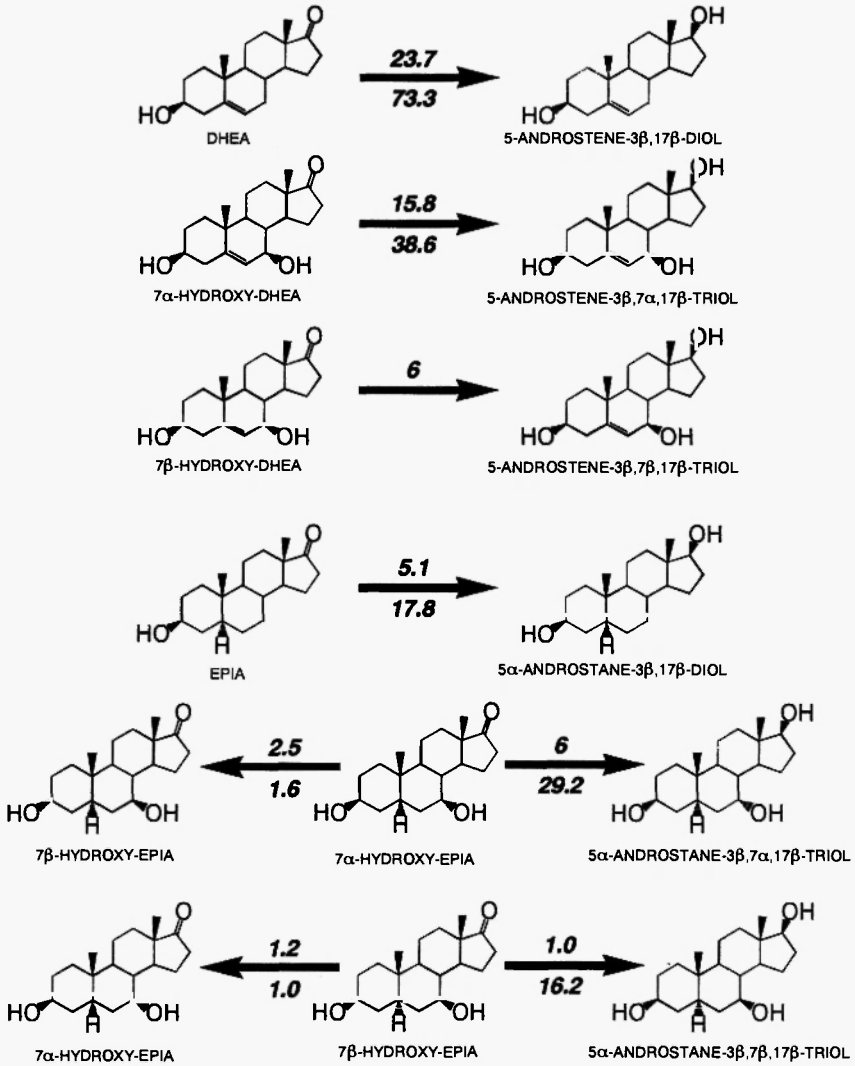

Fig. 3: Transformations of DHEA, EpiA, and related 7-hydroxylated derivatives by pooled human intestinal microsomes /41/. Human intestinal microsomes (0.75 mg protein) were incubated with the [4-^{14}C]-labeled steroid (20 nmol) in 1 ml phosphate buffer (pH 7.4) at 37°C for 20 min. The percent yields of transformation obtained under NADPH and NADH supplementation are given above and below arrows, respectively.

HEPATIC METABOLISM OF DHEA, EpiA AND OF THEIR RESPECTIVE 7-HYDROXYLATED METABOLITES

Many studies have been concerned with the hepatic metabolism of DHEA in several animal models, including rats and mice. Until recently, much less research was devoted to human tissue for DHEA hydroxylation /21,22,37,50/ and for DHEA's effects on human hepatic enzymes /51,52/. No recent report could be found on hepatic EpiA metabolism. However, the hepatic metabolism of these steroids must be assessed, because of the present over-the-counter availability of DHEA generally used by aged individuals, and because of the prospective use of 7-hydroxylated steroids for their anti-glucocorticoid and neuroprotective effects. Therefore, we initiated a study in commercially-available pools of human liver S9 fractions (from male and female donors), and used the previously prepared authentic [4-^{14}C]-labeled steroid substrates /20/. Both cytosolic and microsomal liver enzymes were contained in the S9 fractions, and their incubation with steroid substrates needed supplementation with either oxidized or reduced cofactors (NAD^+, $NADP^+$, NADH, NADPH). After separation, the radio-metabolites obtained were identified with authentic standards after use of gas chromatography/mass spectrometry /53/.

DHEA was best transformed under NADPH supplementation and female samples produced the largest yields. The DHEA metabolites identified are shown in Figure 4: 5-androstene-3β,17β-diol; 16α-hydroxy-DHEA; 7α-hydroxy-DHEA; 7β-hydroxy-DHEA; 7-oxo-DHEA; and another hydroxylated DHEA metabolite that could not be identified because of the absence of a relevant reference. These identifications are in accordance with previous findings in humans /21,22,25,54/, including that of the unknown DHEA metabolite. Both 7α-hydroxy-DHEA and 7β-hydroxy-DHEA incubations led to their NADPH-dependent interconversion and to the $NADP^+$-dependent production of 7-oxo-DHEA, as previously shown by Robinzon *et al.* /41/. None of these steroids was a substrate for 16α-hydroxylation carried out in the liver (Fig. 4).

The EpiA metabolites shown in Figure 5 are: 5α-androstane-3β,17β-diol; 5α-androstane-3,17-dione; 7β-hydroxy-EpiA; 16α-hydroxy-EpiA; and another EpiA metabolite that could not be identified because of the absence of a relevant reference. Provided that the 16α-hydroxylation of EpiA by CYP3A4 could be confirmed, this finding is

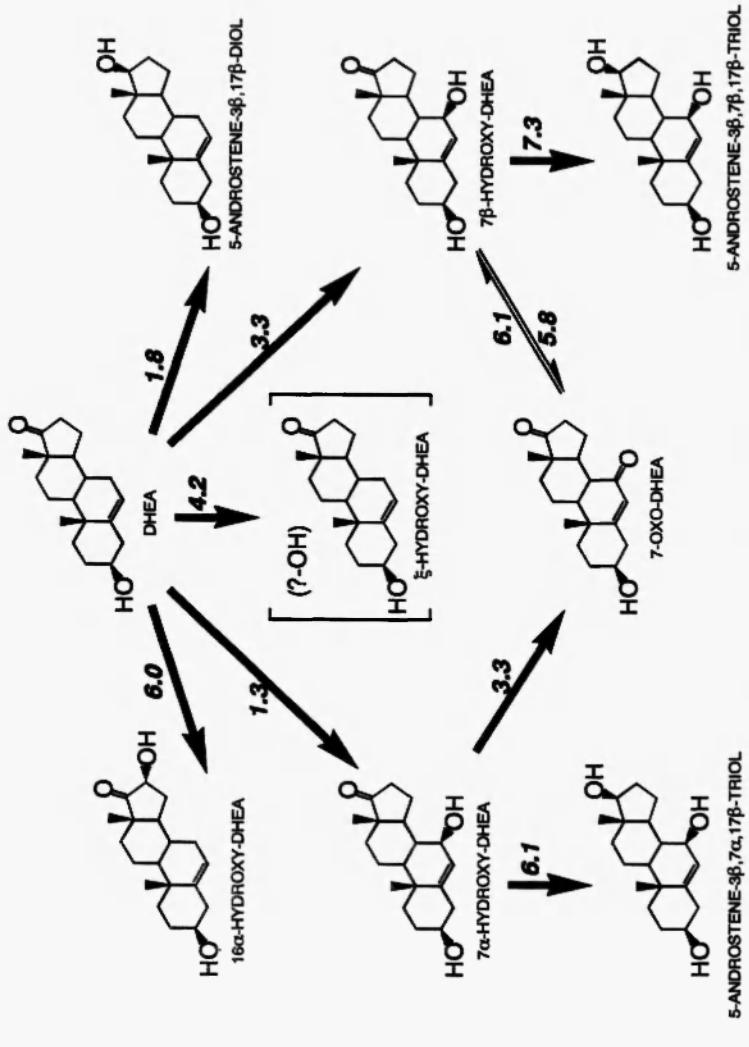

Fig. 4: NADPH-dependent transformations of DHEA and related 7-hydroxylated derivatives by human female liver S9 fractions /53/. The S9 fractions (0.75 mg protein) were incubated with the [4-^{14}C]-labeled steroid (20 nmol) in 1 ml phosphate buffer (pH 7.4) at 37°C for 20 min. The percent yields of transformation are given on each arrow.

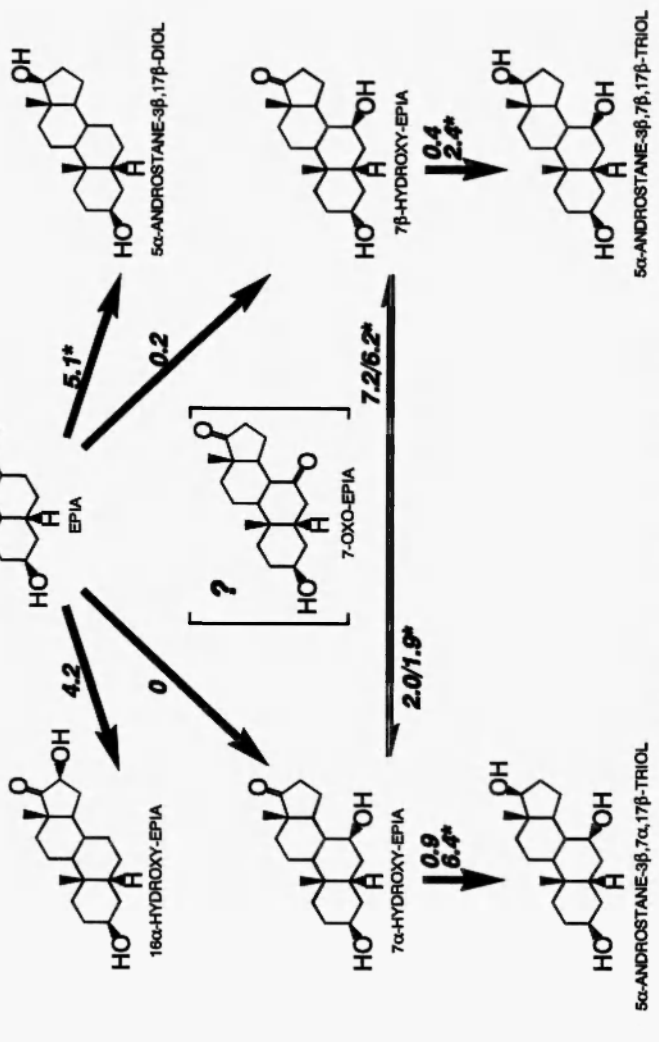

Fig. 5: Transformations of EpiA and related 7-hydroxylated derivatives by human female liver S9 fractions /48/. The S9 fractions (0.75 mg protein) were incubated with the [4-^{14}C]-labeled steroid (20 nmol) in 1 ml phosphate buffer (pH 7.4) at 37°C for 20 min. The major percent yields of transformation were obtained either under NADPH- or NADH(*)-supplementation and are given on each arrow. The NAD^+- and $NADP^+$-dependent productions of 5α-androstane-3,17-dione are not shown here.

in accordance with the higher levels of CYP3A4 found in the human female liver /54/. Nevertheless, it was surprising to find no trace of 7α-hydroxy-EpiA and 7-oxo-EpiA. We have previously reported that other human tissue preparations produce 7α-hydroxylated EpiA /30/ and recently found that the human CYP7B1 expressed in yeast microsomes carried out the 7α-hydroxylation of EpiA with a K_M higher than that of DHEA /14/. The fact that 7β-hydroxy-EpiA was produced instead of 7α-hydroxy-EpiA suggests that one liver P450 carried out the 7β-hydroxylation of EpiA. At present there is no documentation to support a P450-mediated 7β-hydroxylation of EpiA. However, the 7β-hydroxylation of DHEA by CYP3A4 and CYP3A5 is recorded /22,37/ and these CYPs may also carry out the 7β-hydroxylation of EpiA in the liver.

We also observed a 7α/7β interconversion. This is thought to result from an oxidoreduction process catalyzed by 11β-HSD1 using both 7α-hydroxy-DHEA and 7β-hydroxy-DHEA via 7-oxo-DHEA /43/. Our data confirm this phenomenon with DHEA yielding at least equivalent levels of 7α- and 7β-hydroxy-DHEA produced together with large amounts of 7-oxo-DHEA. In turn, 7α-hydroxy-DHEA and 7β-hydroxy-DHEA were both precursors for 7-oxo-DHEA. Concerning 7α-hydroxy-EpiA and 7β-hydroxy-EpiA, their interconversion was obtained without evidence for 7-oxo-EpiA production. It is thus possible that either the 11β-hydroxysteroid dehydrogenase-catalyzed oxidoreduction process reported for 7-hydroxy-DHEA /41,43/ may extend to 7-hydroxylated EpiA substrates, or that an unknown epimerase is involved in the interconversion process. These questions are presently under investigation.

INTERACTIONS WITH 11β-HSD1

The human liver is a well-known target organ for glucocorticoids. Cortisol is the active human glucocorticoid hormone that binds to the glucocorticoid receptor. The human adrenals produce both glucocorticoid (cortisol) and mineralocorticoid (aldosterone) hormones, and cortisol is known to interfere with aldosterone through binding to its receptor. Targets for aldosterone are primarily the kidney and the colon which contain the NAD^+-dependent 11β-HSD2 that inactivates cortisol by an irreversible oxidation into cortisone. Thus, the blood irrigating target organs for glucocorticoids, such as the liver, skin and

brain, contains both active cortisol and inactive cortisone. Here the NADPH-dependent 11β-HSD1 mainly reduces cortisone into cortisol because of their high cellular NADPH/$NADP^+$ ratio /55/.

The decision to examine 7-oxygenated 5-androstenes as substrates for human 11β-HSD1 was influenced by several reasons: i) several studies in animals and humans inferred that the 11β-HSD1-mediated oxidoreduction of 7-oxygenated steroid substrates could occur /41,42, 56,57/; ii) the 7α-hydroxy (axial) position in steroids closely resembles the 11β-hydroxy position /38/; and iii) the oxidoreduction process leading to the production of 7β-hydroxy-DHEA was thought to have involved a putative reductase specific for the 7-oxo-DHEA intermediate /41/.

The human 11β-HSD1 was produced in microsomes of transformed yeast and its activities were examined on the oxidation of [4-^{14}C]-labeled cortisol, 7α-hydroxy-DHEA and 7β-hydroxy-DHEA, and on the reduction of [4-^{14}C]-labeled cortisone and 7-oxo-DHEA substrates. Either an $NADP^+$-regenerating system or an NADPH-regenerating system was used. Oxidations into cortisone and 7-oxo-DHEA, and reductions into cortisol, 7α-hydroxy-DHEA, and 7β-hydroxy-DHEA, were obtained, and the kinetic parameters for each step were measured /43/. It is possible to justify that both cortisol and 7α-hydroxy-DHEA were oxidized because both 11β- and 7α-secondary alcohols are in axial positions which overlap after rotation of one steroid molecule /58/ thus giving rise to cortisol and 7α-hydroxy-DHEA equivalent structures for 11β-HSD1. In contrast, the 7β-hydroxy-DHEA bears its 7-hydroxyl as an equatorial hydrogen substitute, and both rotation and flip of the molecule are necessary for meeting the structural requirements of cortisol /58/. Presently, the specificity of steroid oxidoreductases for the recognition of a single steroid epimer is basic to their nomenclature and function, and the 11β-HSD1-catalyzed oxidation of 7β-hydroxy-DHEA into 7-oxo-DHEA stands as contrary to that concept. In fact, the yields of 7-oxo-DHEA obtained from 11β-HSD1-mediated oxidation of 7β-hydroxy-DHEA were much greater that those from 7α-hydroxy-DHEA. Therefore we conclude that human 11β-HSD1 is a steroid oxidoreductase able to use and produce 7α- and 7β-hydroxy-DHEA through an oxidoreduction process generating the 7-oxo-DHEA intermediate. A schematic representation for the 11β-HSD1 mechanism is given in Figure 6.

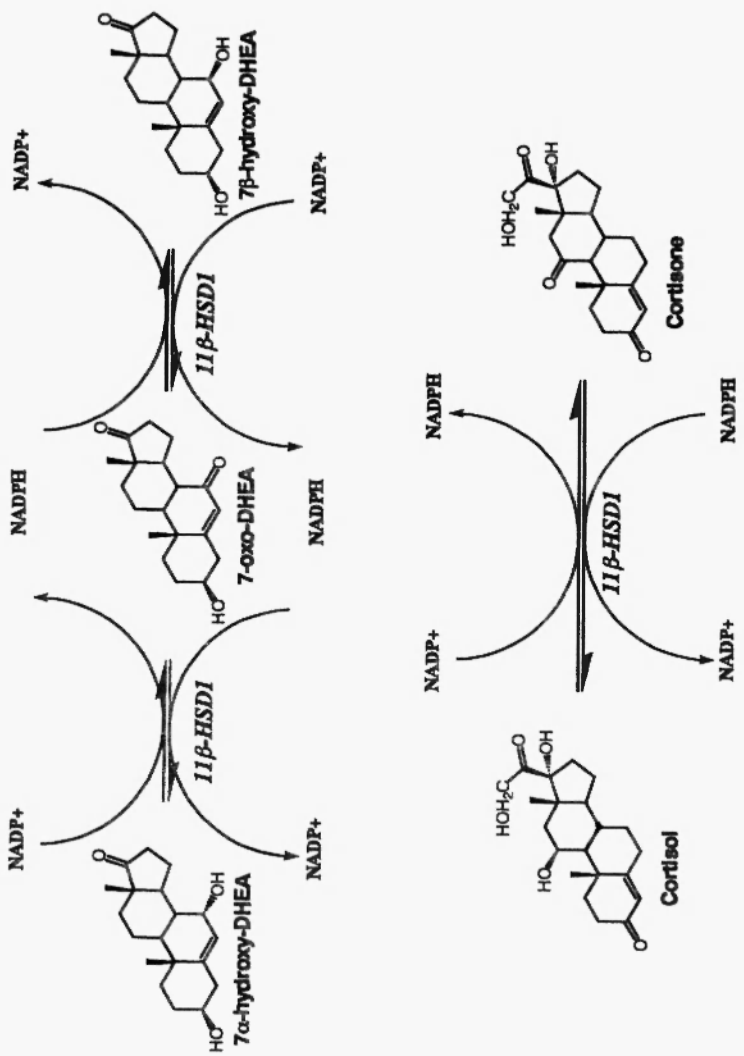

Fig. 6: Human 11β-HSD1 catalyzes both cortisol-cortisone oxidoreduction and the interconversion of 7α- and 7β-hydroxy-DHEA through the 7-oxo-DHEA intermediate.

This information is important because of the following: i) it demonstrates that a single enzyme accepts different epimeric steroid substrates for oxidation; ii) 11β-HSD1 is responsible for the interconversion of 7α- and 7β-hydroxy-DHEA via the 7-oxo intermediate; iii) 11β-HSD1-mediated interconversion may occur on other steroids, such as 7α-hydroxycholesterol and 7β-hydroxycholesterol /56,57,59/; and iv) 11β-HSD1's involvement both for the generation of cortisol from cortisone and for the oxidoreduction of 7α- and 7β-hydroxy-DHEA substrates might explain the anti-glucocorticoid effects obtained *in vivo* with the latter /60,61/. The anti-glucocorticoid mode of action could not be explained by interference with the human glucocorticoid receptor activation and trafficking /62/.

CONCLUDING REMARKS

The metabolism of steroids and hormone receptors has been extensively studied for years. However, at present, and among the investigators concerned with DHEA, there are only a few teams who investigate aspects of steroid metabolizing enzymes. As DHEA proved to be a poor trigger for anti-glucocorticoid effects, one of its metabolites was suspected to play a role. That is, the 7α-hydroxylated derivative was thought to be the native anti-glucocorticoid produced in target organs for glucocorticoid hormones /63/. The mechanism of its anti-glucocorticoid action could not occur by the conventional mechanism of glucocorticoid action via a specific receptor in target cells /62/, but could rather involve interference with 11β-HSD1 that usually transforms inactive cortisone into the active cortisol /43/. Two key concerns result from this: one is the level of circulating DHEA and its sulfated form which may turn into DHEA through the action of sulfatases commonly found in tissues; the second involves the activity of the CYP7B1 responsible for the production of 7α-hydroxy-DHEA. As DHEA levels decrease with ageing, there is obviously less DHEA available for the 7α-hydroxylating enzyme and, therefore, decreasing levels of circulating 7α-hydroxy-DHEA /64/. One of the difficulties encountered in deciphering 7α-hydroxy-DHEA's mechanism of action was the absence of any systemic effect triggered by this steroid. This may be explained by its incapacity to interfere with the binding of the glucocorticoid hormone to its receptor and by its apparent autocrine or paracrine mode of action. Thus intracellular 7α-hydroxy-DHEA pro-

duction and its levels require investigation instead. At present, several questions are in need of answers: Does CYP7B1 activity decrease with age? Use of the monoclonal IgM that we produced for its specific detection /27/ is a tool that should provide efficient help for answers to this question in several human tissues. Could CYP7B1 polymorphism explain the wide variations between individuals of the same age? Our comparison of CYP7B1 sequences in mice, rats and humans with the resulting K_Ms in the 7α-hydroxylation of DHEA and pregnenolone led us to suspect that polymorphism may generate widely different 7α-hydroxylating capacities /26,65/.

Finally, other cellular aspects need to be considered, and these concern the availability of reduced and oxidized cofactors. The name ergosteroid coined by Lardy *et al.* for several 7-oxygenated-steroids includes 7α-hydroxy-DHEA /66/. When administered to rats in food, these steroids induced a significantly increased activity of the malic enzyme and *sn*-glycerol-3-phosphate dehydrogenase of the liver. These enzymes are involved in electron transport from the tricarboxylic cycle, and generate a large part of cellular NADPH /67/. Whether these enzyme inductions resulted from a direct action by the 7-oxygenated steroids or from their anti-glucocorticoid effect in the liver of the living rat has not yet been established. All research along these lines in normal, aged and diseased organisms should yield valuable results and knowledge for use towards better human health.

It is of interest to note that the metabolic transformation of hormones is a key to the regulation of endocrine activities. This was exemplified by the 5α-reduction of testosterone leading to 5α-dihydrotestosterone and its binding to the androgen receptor in target tissues. The anti-glucocorticoid effects proposed through 7α-hydroxy-DHEA production and interference with 11β-HSD1 is now another example. Steroid metabolism is rapid and at no cost to the organism when compared to all putative gene-dependent factors and peptides generally proposed for the control and activation of steroid hormone actions. According to Ocham's razor, the simplest mechanism is generally the most probable.

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