MINIREVIEW: 16α-HYDROXYLATED METABOLITES OF DEHYDROEPIANDROSTERONE AND THEIR BIOLOGICAL SIGNIFICANCE

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16α-Hydroxydehydroepiandrosterone is the precursor of fetal 16α-hydroxylated estrogens, the main phenolic steroids in pregnancy. For years their serum levels have been used as biochemical markers of well being of the fetus. In adults, however, increased levels of 16-hydroxylated estrogens were put in relation to the risk of cancer and, more recently, to some systemic autoimmune diseases. With respect to immunomodulatory effect of dehydroepiandrosterone and its metabolites, of which 7-hydroxysteroids formed by a concurrent reaction to 16-hydroxylation, are believed to be even the more immunoprotective species, it may be of interest, what is the true role of 16α-hydroxydehydroepiandrosterone and other 16-hydroxysteroids of 3β-hydroxy-5-ene series. The present knowledge on the latter metabolite was summarized and discussed.

Recently we have published a mini-review on 7-hydroxylated metabolites of dehydroepiandrosterone (DHEA) and its precursors in biosynthetic route, as immunomodulatory and immunoprotective agents, acting predominantly locally in the sites of injury (Hampl et al.1997). A concurrent metabolic reaction of DHEA, its precursors and metabolites is the hydroxylation at carbon 16, resulting in the formation of 16α- or 16β-hydroxylated metabolites (Baulieu 1996; Baulieu and Robel 1998). The main metabolic reactions of DHEA including the sites of 16-hydroxylation on steroid molecule are shown in Fig. 1.

16-Hydroxylation and pregnancy

From the physiological point of view 16α- and 16β-hydroxylation of steroids is an important enzymatic reaction and a prerequisite for the formation of 16-oxygenated estrogens, of which the most important is estriol (1,3,5(10)-estratriene-3,16α,17β-triol), the main estrogen in pregnancy. For years the levels of this metabolite have been used as a marker of well being of the fetus.

According to the concept introduced by Diczfalusy in sixties and known as the fetoplacental unit, DHEA, as a sulfate the most abundant circulating steroid, is at first hydroxylated at 16α-positition in fetal liver and then, following passage across the fetoplacental barrier, is hydrolyzed by steroid sulfatase and finally aromatized in placenta (Goodyer and Branchaud 1981; Newby et al. 1998). Generally, insufficient production of placental estrogens in midpregnancy may lead to risk or even pathological development of the fetus. The reason of low estrogen production have been searched either in decreased of DHEAS supply, lowered activity of 16α-hydroxylation, and sulfatase or aromatase deficiency, respectively. Several reports brought evidence that sulfates deficiency is the major cause of estrogen insufficiency (Wilmot et al.1988; Glass et al. 1998), though it must not be true for all instances as demonstrated by Newby et al. (2000) in Down's syndrome pregnan-

cies, where the main cause was the diminished supply of the fetal DHEAS.

16-Hydroxysteroids in biological fluids

16-Hydroxylated metabolites of DHEA have been detected as early as in fifties in umbilical blood of newborns (FOTHERBY et al. 1957). Later several reports appeared on the isolation and identification of various 16α- or 16β-hydroxysteroids in urine of children and adults with various endocrine disorders as well an in experimental animals (COLAS et al. 1964; REYNOLDS 1965; SHACKLETON et al.1965, 1968; CLEARY and PION 1968). More recently, relatively high concentrations of micromolar range of 16-hydroxysteroid sulfates have been detected in breast cyst fluid (see below the section on "16-Hydroxylated estrogens: risk factors for cancer?")

Enzymology of steroid 16-hydroxylation

16-Hydroxylation of estrogens, androgens and their precursors is not confined to the liver or adrenal cortex, but it occurs also in several other tissues. The literature until the first half of sixties has been comprehensively reviewed by DORFMAN and UNGAR (1965) and later by BREUER (1967). More detailed studies from seventies and later of the respective enzymes operating in the organism revealed their various isoforms.

Proteins responsible for 16α- or 16β-hydroxylation of steroids are commonly classified as EC 1.14.14.1 enzyme named unspecific monooxygenase, belonging to cytochrome P450 family, acting on paired donors with incorporation of molecular oxygen with reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen. To date (July 14, 2000) not less than 244 genes were described and characterized of which 23 were found in humans, encoding various proteins and isoenzymes, differing in substrate specificity and tissue localization. For information see e.g. Internet address *www.expasy.ch/cgi-bin/enzyme/*. In concert with another enzyme NADPH-ferrihemoprotein reductase (EC 1.6.2.4) they form a system in which NADPH supplies two reducing equivalents. With respect to steroid 16α-hydroxylation, two enzymes are of particular importance, namely CYP 3A4 present in human liver microsomes and CYP 1A1

from various tissues including brain (LACROIX et al. 1997; NIVA et al. 1998). A typical substrate for the former enzyme is nifedipine (Verapamil), a well-known vasodilatant (NIVA et al. 1998). In addition, under certain conditions 16α-hydroxylating activity may exhibited even by other steroid metabolizing enzymes, as demonstrated e.g. by P450- dependent 17α-hydroxylase expressed in tumor cells transfected with its gene (SWART et 1993).

16α-hydroxylated metabolites of DHEA: what is their biological significance ?

The physiological role of 16α-hydroxydehydroepiandrosterone (16α-OH-DHEA) and its 16α-hydroxylated precursors is not known. To our knowledge the only reports on the levels of 16α-OH-DHEA, its 16α-hydroxylated precursors and their sulfates deal with prenatal or early neonatal period and with women during labor (FURUYA et al. 1976; CHANG et al. 1976; SHIBUSAWA et al. 1978; DEN et al. 1979; TAGAWA et al. 1997). The most common material for determination of these steroids was umbilical blood, in which relatively high levels of tens nanomols of unconjugated 16α-OH-DHEA were detected. Perhaps the only paper dealing with physiological levels of 16-OH-DHEA during the life span is that concerning 16β-hydroxyisomer of DHEA (SEKIHARA et al. 1976). Surprisingly, elevated concentration of this metabolite has been found in conjugated fraction of urine from patients with essential hypertension (NOWACZYNSKI et al. 1977). However, no explanation of this phenomenon was given.

16-Hydroxylated estrogens: a risk factors for cancer ?

Much more reports were devoted to possible physiological and pathophysiological role of 16α-hydroxylated estrogens. It was demonstrated that in mice 16α-hydroxylated estrogens were closely associated with mammary tumors (BRADLOW et al. 1985, 1986a). Several studies have been undertaken to test whether the concentrations of 16α-hydroxylated estrogens and their urinary metabolites do correlate with incidence of breast and endometrial cancer in women, but the results were not unequivocal (FISHMAN et al. 1984; BRADLOW et al. 1986b; URSIN et al. 1997, 1999). It has been suggested that cancerogenicity of natural
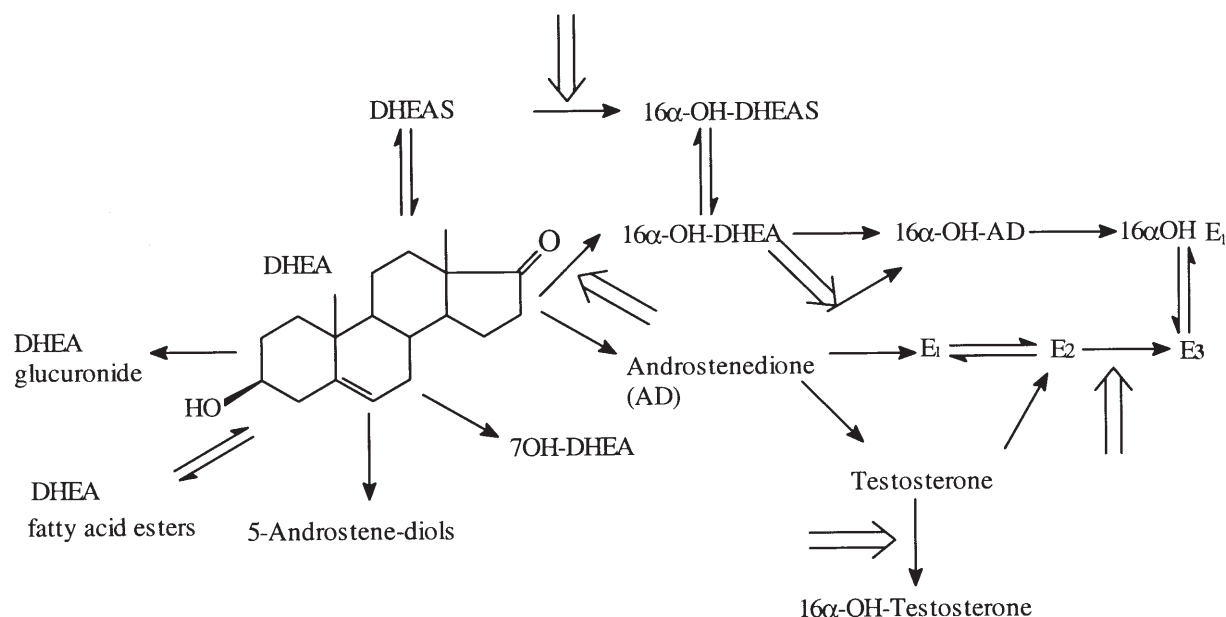
Fig. 1 Metabolism of DHEA with depicted sites of 16-hydroxylase action (double arrow). Abbreviations: DHEA, DHEAS, dehydroepiandrosterone and its sulfate; AD, androstenedione; E_1, E_2, E_3, estrone, estradiol, estriol

16α-hydroxylated estrogens could be caused by their easy formation of covalent adducts with protein macromolecules (as e.g. with estrogen receptors) (BRADLOW et al. 1986a; BHAVNANI 1998). This reaction does not proceed with 16α-hydroxylated equine estrogens with B-unsaturated ring which are to be avoiding of carcinogenic properties (BHAVNANI 1998).

As mentioned above, 16α-OH-DHEAS, along with estriol sulfate, were determined in breast cystic fluid (BCF) as potential tumor markers. The individual values of 16α-OH-DHEAS in BCSF showed a broad range from 0.04 to 2.8 μmol/l. A positive correlation has been found between both the 16α-OH-DHEAS and estriol sulfate on one side and the concentration of potassium which is known to be increased in patients with a high risk of breast tumor development on the other (RAJU et al. 1989).

16-Hydroxylated estrogens in autoimmune diseases

In addition, significantly increased serum levels of 16α-hydroxylated estrogens have been found in patients with typical systemic autoimmune diseases as systemic lupus erythematosus or rheumatic arthri-

tis (LAHITA et al. 1981, 1985; MERRIL et al. 1996). It is of interest that in these patients low levels of DHEA have been found, too (MERRIL et al. 1996). With regard to recent findings of immunomodulatory and immunoprotective role of the latter steroid (KALIMI et al. 1994; WATSON et al. 1996; ARANEO and DAYNES 1995) it would be of extreme interest to find out what is the role of the metabolic route leading to 16α-hydroxylated DHEA metabolites. Recently it has been shown that another DHEA metabolites, namely its 7α- and 7β-hydroxylated steroids, which are also present in the circulation, may serve as locally active immunoprotective agents, in some situations even more potent than DHEA itself (HAMPL et al. 1997; LAFAYE et al. 1999). One may speculate that a concurrent reaction, namely 16α-hydroxylation may result in a formation of compounds with an opposite effect.

Open questions

Taking together, since DHEA and its precursors (pregnenolone and 17α-hydroxypregnenolone) are the first targets for 16α-hydroxylation, it seems worth to investigate their levels at physiological and patho-

physiological conditions in order to fill up the gap in our knowledge. A special respect should be paid to breast and other cancers of female reproductive tract, as well as to the patients with autoimmune diseases.

The only immunoanalytical methods available for determination of these steroids in body fluids are from midseventies. The radioimmunoassays described used immunogens prepared from haptens attached to the carrier protein via 3β-hydroxygroup, which is inherent to naturally occurring steroids (Furuyama et al. 1976; Chang et al. 1976; Shibusawa et al. 1978; Den et al. 1979; Tagawa et al. 1997). Thus the methods suffered from a poor specificity. Therefore, the first task is to develop a reliable and specific method for determination of 16α-OH-DHEA. Our laboratory is before launching a specific immunoassay for this substance, based on antisera constructed against haptens prepared via the position 7 and 19 of the steroid molecule. After having it in our hands, we believe to shad the light on at least some ot the above outlined questions.

Acknowledgement

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