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Special article

Tissue specificity: the clinical importance of steroid metabolites in hormone replacement therapy

Doris M. Gruber *, Johannes C. Huber

Department of Gynecological Endocrinology and Reproductive Medicine, Clinic for Gynecology and Obstetrics, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

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Abstract

Metabolic activations or inactivations of estrogens, progesterone and androgens are important steps towards the understanding of the physiological and the pathological effects of these hormones in the female organism. Analysis of the tissue specific metabolic pathways of sex steroids will result in a better understanding of successful hormone replacement therapy on the one hand and of the occurrence of steroid hormone related side effects on the other hand. In this contribution we analyse the different mechanisms involved in the synthesis of tissue specific metabolites and discuss the therapeutical importance of these metabolites in hormone replacement therapy.

Keywords: Hormone replacement therapy; Metabolite replacement therapy; Progesterone; Androgen; Estrogen

1. Introduction

The clinical use of selective estrogen receptor modulators and tissue specific compounds raises the question for the clinical value and pharmacological relevance of organ specific metabolites. Sexual steroid hormones are usually synthesized by specific glands, they are metabolised peripherally and the total range of elicited biological effects can be influenced by specific biological activities of some of these metabolites. This aspect of the tissue specific adaptation to the physiological environment may become of interest in hormone replacement therapy using synthetic compounds, because these compounds are biochemically different to the naturally secreted glandular steroid hormones and thus have different metabolites.

In endocrinology the most famous and related example for replacement therapy with metabolites playing a role, is thyroid substitution therapy. Triiodothyronine (T3), the main metabolite of

* Corresponding author. Tel.: +43-1-404002816; fax: +43-1-404002817.

E-mail address: doris.gruber@akh-wien.ac.at (D.M. Gruber).

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thyroxine (tetraiodothyronine, T4), can be used for successful substitution although this metabolite has its own pharmacological profile, slightly different from thyroxine. Triiodothyronine is derived from the original hormone thyroxine, which is de novo synthesised in the thyroid gland and is metabolised in the liver, muscles, fibroblasts, heart, brain and nearly every other human tissue. Triiodotyronine and thyroxine are different in their biological and pharmacological aspects, which is the reason why in thyroid substitution therapy the combined substitution of thyroxin and triiodothyronine — the replacement of the original hormone and its metabolite — has advantages over thyroxine replacement therapy alone. In a recent study the authors conclude that the ideal regimen, when thyroid-gland function is absent or nearly absent, might consist of triiodothyronine in combination with enough thyroxine to ensure euthyroidism [1].

2. Clinical importance of progesterone metabolites

Pure progesterone is produced in the corpus luteum, and metabolised mainly in the liver, but also in the brain and other tissues. In the brain some of the metabolites can act as neurosteroids, they are important for nutrition and function of cells in the central nervous system.

Progesterone can have several metabolic fates, there is 5α- and 5β-reduction, 20α- and 20β-reduction and a 6β-, 16α-, 17α- and 21-hydroxylation [2,3]. Reduction of progesterone in the C5 position results in metabolites with a 5α- and 5β-configuration. 5β-reduced metabolites are clinically important in pregnancy, underlining the hypothesis, that metabolites exert distinguished functions in different physiological conditions such as ovulation and the premenstrual status.

The 5α-reduction of progesterone takes place in hepatocytes as well as in extrahepatic sites. Two different 5α-reductases, representing two different gene products, have been cloned, one of which is primarily responsible for generation of 5α-reduced androgens. It has been estimated that 5α-reduction accounts for 50% of progesterone metabolism in the liver [2]. These 5α-reduced metabolites are conjugated (principally as sulphates), secreted into bile, and ultimately excreted in the feces [4].

Majewska et al. [5] reported that both 3α-hydroxy-5α-tetrahydroprogesterone (3α-OH-5α-THP, allopregnanolone), a metabolite of progesterone, and 3α-OH-5α-tetrahydrodesoxycorticosterone (3α-OH-5α-THDOC), are potent barbiturate-like ligands of the gamma amino butyric acid receptor, the GABA-A receptor. They are 700 and 1000 times more effective to bind at the chloride channel than the barbiturate pentobarbital. They also determined that 3α-OH-5α-THP stimulates the uptake of ionic chloride into brain vesicles in the same way as barbiturates and GABA. When the GABA-A receptor is activated by binding of GABA or a GABA agonist, chloride ions move through the channel, leading to hyperpolarisation of the cell membrane. The hyperpolarisation increases the threshold for action potentials, thereby inhibiting the responsiveness of the cell. The progesterone effect appears to be stereospecific and is now known to result in agonistic (e.g. sedative), partial agonistic, or antagonistic (e.g. convulsive) effects on the receptor.

Progesterone and its neuroactive metabolites are likely to have physiological and pathophysiological significance during the menstrual cycle and pregnancy [5] Indeed these neuroactive steroids have been proposed to cause the fatigue, experienced by some women during pregnancy and in the premenstrual syndrome (PMS) [6].

Subjects with PMS manifested lower levels of the anxiolytic 3α-OH-5α-reduced progesterone metabolite allopregnanolone in the blood in the luteal phase when compared with controls. Diminished blood concentrations of allopregnanolone in women with PMS may lead to an inability to enhance gamma aminobutyric acid-mediated inhibition during states of altered central nervous system excitability such as ovulation or physiologic or psychological stress. The lowered metabolite levels could contribute to the genesis of various mood symptoms, such as anxiety, tension and depression as observed in PMS patients [6]. The involvement of reduced progesterone metabolites in regulation of GABA-A receptors may represent an important link between endocrinologic events and the central nervous sys-

tem (CNS) [7]. In contrast to that, Schmidt et al. found no significant correlations between the severity of mood and behavioral symptoms and plasma levels of progesterone, allopregnanolone, and the epimeric 3α-OH-5β-pregnan-20-one (pregnanolone) [8]. Steroid levels in the serum do not always reflect the pharmacological situation in different tissues, such as in the CNS. This may be an explanation for the contradictory finding of Schmidt et al. [8], as there were no differences in the study population. Progesterone and allopregnanolone can be synthesised in the CNS, as evidenced by the fact that these compounds are found in the CNS even after combined adrenalectomy and ovariectomy [9]. Not all but the major enzymes required for their synthesis from cholesterol also have been found in the CNS [9]. Progesterone from the peripheral circulation has been shown to accumulate in various brain regions and can be converted to 5α-dihydroprogesterone and allopregnanolone. Peripherally administered 5α-reduced progesterone metabolites also accumulate in various brain regions, and 5α-dihydroprogesterone and allopregnanolone have been shown to be active as neuroendocrine modulators of FSH and LH secretion [10].

Depression, mood disorders and anxiety are important clinical features in the menopausal syndrome and can partially be treated by estrogen replacement therapy. The pyschotropic effect of progesterone and its metabolites should be reconsidered in cases where classical combined replacement therapy cannot alleviate depressive feelings, as the synthetic progestin could even intensify mood disorders. Whether synthetic progestins can exert the same direct action on the CNS membrane receptor as natural progesterone is not yet clear. It is unlikely, that synthetic steroids can be converted into neuroactive steroids.

The onset of labour in many animal species is associated with progesterone withdrawal followed by an increase in circulating oxytocin. It remains controversial whether in human labour these processes are similar. One of the actions of progesterone in the rat is to reduce oxytocin binding to its receptor. Although progesterone does not have this effect on the human receptor, a metabolite (5β-dihydroprogesterone) reduces oxytocin binding to the human (but not rat) receptor in cultured cells [11]. This suggests a link between progesterone, via an active metabolite, and the oxytocin receptor in the human. There also seems to exist an evolution in the balance of endocrine secreted steroids and their metabolites in respect to different physiological functions. Thornton et al. found that 5β-dihydroprogesterone reduces oxytocin-receptor binding or intrinsic receptor activity during human pregnancy. Assuming that the 5β-dihydroprogesterone effect is specific for the oxytocin receptor, reduced synthesis or enhanced degradation of 5βDHP could enable oxytocin-receptor-signal transduction leading to contraction [12].In non pregnant females 5β-reduced metabolites are pyrogenic and also induce heme synthesis in liver by increasing δ-aminolevulinic acid synthetase activity as do 5β-reduced androgenic metabolites [13]. This aspect may also become of clinical importance in postmenopausal patients.

2.1. Clinical importance of androgen metabolites

The biological activity of testosterone is increased by 5α-reductase, an enzyme that reduces testosterone to dihydrotestosterone (DHT), which is three times more potent than testosterone. Its production occurs in the skin, in the hair follicle and in the male reproductive tract. Androgens are also metabolised in the liver and other tissues to relatively inactive steroid sulfates and glucuronides of 3α-androstanediols (5α-androstane-3α,17β-diol), androsterone (5α-androstane-3α-ol-17-one) and etiocholanolone (5β-androstane-3α-ol-17-one). The enzyme aromatase changes the biopotency of testosterone by converting it to estradiol, which has functions in the brain, breast, liver, bone and other tissues. DHT cannot be aromatised, an interesting aspect in androgen replacement therapy.

Considerable attention has been focused on the role of androgens and estrogens in modulating abdominal fat distribution. In clinical studies, correlations between testosterone levels and visceral fat accumulation have been shown in both men and women [14]. On the one hand an increase of visceral and truncal fat is observed in patients

with ovarian hyperandrogenism and PCO syndrome [15]. On the other hand, it is well known that hypoandrogenemia in postmenopausal women results in increased subcutaneous abdominal fat leading to overweight [16,17]. Marin et al. [18] demonstrated in men that administration of testosterone decreased visceral fat without significantly affecting subcutaneous fat or overall body composition. Lovejoy et al. [19] demonstrated that the administration of exogenous androgens modulates body composition in obese postmenopausal women and independently reduces visceral and subcutaneous fat. Furthermore, it is known that androgen treatment can decrease abdominal fat as the expression of lipolytic β-adrenergic receptors is positively regulated by testosterone [20] and, therefore, enhancement of lipolysis can be expected. As testosterone can be converted to estrogens which counteracts the lipolytic effect, the nonaromatisable DHT seems to be a better candidate for androgen replacement therapy [21].

Leptin, the product of the Ob gene, expressed in adipocytes acts as a signalling factor from the adipose tissue to the CNS, regulating food intake and energy expenditure. It has been reported that circulating leptin levels are higher in women than in men, even after correction for body fat. This gender-based difference may be conditioned by differences in the level of androgenic hormones. In vitro exposure to DHT resulted in a significant reduction of leptin secretion in samples taken from women, with no effect on male adipose tissue. Testosterone, the original hormone did not have an effect on leptin secretion [22]. The metabolite DHT is commercially available for male patients, but is rarely used for androgen replacement therapy in females.

DHT has also immunological properties; direct exposure of T-cells to DHT in vitro was found to reduce the secretion of gamma interferon; exposure of T-cells to androstanedione or testosterone resulted in no change in the biosynthesis of these lymphokines. Macrophages are able to express the enzyme 5α-reductase and are competent to metabolise testosterone to DHT. By incubating bone marrow macrophages with testosterone, DHT was produced and interferon gamma production ceased. DHT, similar to other steroid hormones like dehydroepiandrosterone, may play an important role in lymphokine expression and regulation in vivo [23]. A systemic administration of DHT appears to have direct effects on the expansion of Th2 cell populations with subsequent restoration of normal immune response [24].

DHT also seems to be important in Sjögren syndrome. A critical level of DHT is necessary to maintain lacrimal gland structure and function, and a decrease beyond this androgen level could trigger lacrimal gland apoptosis and necrosis. This process could be effectively prevented by DHT substitution [25].

Dehydroepiandrosterone (DHEA) unconjugated or as its sulphate (DHEAS) is the major secretory steroidal product of the adrenal gland. Despite the abundance of DHEA and DHEAS their physiological role has remained unknown [26]. In vitro and in vivo data suggest estrogen and androgen like effects, depending on the estrogen and androgen serum levels respectively [27]. Also the DHEA metabolite 5-androstene-3β, 17β-diol (ADIOL) has both androgenic and estrogenic effects in human myometrial tissue and in mammary glands [28]. In these cells ADIOL is 500 times more potent as an inhibitor of estrogen binding than DHEA. The estrogen like effects of ADIOL, which is structurally closer to estradiol than to estrone or estriol, are observed at physiological concentrations in breast cancer cells [29]. In premenopausal women DHEA is either an estrogen antagonist, perhaps through the competitive binding of its metabolite ADIOL and estradiol to the estrogen receptor, or an androgen through its metabolism to androstenedione and testosterone. The physiological action of DHEA, directly or via ADIOL depends on the hormonal milieu and DHEA or its metabolite is involved in body fat distribution, insulin resistance, cardiovascular disease and even hormone dependent tumor growth [30].

2.2. Clinical importance of estrogen metabolites

The direction of the metabolism of estradiol in man has a profound impact on the nature of the biological responses to the hormone.

The major metabolic products of estrogens are the catechol estrogens. The principal natural estrogen, estradiol, is transformed in cells into the following main components: estrone (E1), 2-hydroxyestrone (2-HE), 4-hydroxyestrone (4-HE), and 16α-hydroxyestrone (16α-HE). The initial oxidation of the 17β-hydroxy group yields estrone. This steroid is subsequently metabolized mainly through either of two alternate hydroxylation pathways: hydroxylation at the C-2 or the 16α position. These hydroxylations are of particular interest in that they constitute competing reactions whose products are themselves active compounds characterized by markedly different biological properties. The estrogenic compounds can react directly with DNA or can be further metabolised. What actually damages DNA and leads to tumor growth may be the products of 4-HE. Numerous enzymes can change 4-HE into compounds called 3,4-semiquinones and 3,4-quinones. These compounds bind to DNA, form adducts and have the strong potential to create gene mutations [31]. Most researchers agree that 2-HE is not dangerous, because it fails to show up as a mutagen in cell culture studies or as a carcinogen in animals [32]. Instead 4-HE and 16 α-HE are highly suspicious to be involved in carcinogenesis.

Estrogen metabolism takes place in the liver as well as in other extrahepatic sites where the product is primarily 2-HE. The ratio of 2-hydroxylated to 16α-hydroxylated derivatives varies widely in a number of different physiological states. 2-Hydroxylated derivatives predominate in individuals with anorexia, whereas 16α-hydroxylated derivatives predominate in obese individuals [33]. This bifurcation in the pathway of estrogen metabolism may have significance because the metabolites of each pathway have different biological properties; the 2- and 4-catechol estrogens are devoid of peripheral estrogenic activity, while metabolites with a 16α-hydroxy group sustain the estrogenic activity of the parent hormone [34]. 16α-HE also has a low affinity for sex hormone-binding globuline, thereby increasing the potential of this metabolite to effect target tissues. 16α-Hydroxylated compounds (including estriol) are themselves potent estrogens and changes in the serum levels may have important consequences on the etiology of breast cancer [35].

Longcope et al. [36] found that the extent of 17β-estradiol metabolised by 2-hydroxylase oxidation can be increased when the percentage of calories consumed as fat is as low as 25%. Differences in estrogen metabolism are associated with several known risk factors for breast cancer and there is evidence that environmental circumstances seem to influence estrogen metabolism [37]. The drug nicotine has also an influence on estrogen degradation [38]. Cigarette smoking in women induces an increase in estradiol 2-hydroxylation and, if anything, reduces the risk for breast cancer. This irreversible metabolic pathway yields 2-hydroxy-estrogens, which possess minimal peripheral estrogenic activity and are cleared rapidly from the circulation.

2-Hydroxy-estradiol is further metabolised and methylated to 2-methoxyestradiol (2-ME), which is important because 2-ME is an anti-angiogenic metabolite and a mitose-inhibitor in pre-cancer cells [39]. The growth inhibition is due to mitotic arrest and apoptosis. These effects are reminiscent of those induced by taxol, and appear to be mediated by inhibition of microtubule dynamics [40]. Therefore the question should be raised, whether 2ME is the endogenous estrogen metabolite that is capable of inhibiting angiogenesis supporting carcinogenesis.

Studies have also shown that 4-HE is produced in the tissues where estrogen-linked cancers develop. Most estrogen metabolism takes place in the liver, where the product is primarily the safe 2-HE. In 1996 it was discovered that an enzyme known as cytochrome P-4501B1 converts 17β estradiol into 4-HE. This enzyme is more abundant in the breast than in tissues not prone to estrogen-linked cancers [32]. There are differences in the effects of using transdermal or oral estradiol substitution which have clinical implications. Following oral administration of conjugated estrogens, sufficiently high estrogen levels are reached, since absorption is accelerated. The estrone/estradiol ratio, which is 1:2 in fertile women and 2:1 in postmenopausal women, increases to 4:1 after oral treatment. However this first pass effect should not be regarded as a drawback, since

it results in the formation of estrone/estrone sulfate reservoir. Bypassing of this first pass-effect by topical application leads to higher estradiol and lower estrone levels. The estrogen levels are to a certain extent dependent on the dose applied topically and are also influenced by the area of application.

If there indeed is a highly sophisticated individual steroid metabolism that is important for some of the physiological functions of sex steroids, this aspect may become of great interest for estrogen replacement therapy.

3. Conclusions

One of the compliance-limiting factors in HRT is the diversity of side effects that might occur in some women. The explanation for these effects may be partially found in the way exogenous and endogenous steroids are metabolized within the female organism. Because the glandularly secreted hormone is not always the acting compound, specific tissue metabolites can be of tremendous importance for the final hormonal action in target tissues. The action of steroid metabolites in HRT will gain more and more attention in the future. Metabolites will on the one hand either be used for specific aspects in substitution therapy or on the other hand their formation must be prevented due to their potentially deleterious effects in the human organism.

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