REVIEW

The Origin of 7α-Hydroxy-Dehydroepiandrosterone and Its Physiological Role: a History of Discoveries

L. STÁRKA

1Institute of Endocrinology, Prague, Czech Republic

Received February 14, 2017
Accepted March 20, 2017

Summary

Nearly 60 years has elapsed since the first isolation and identification of 7α-hydroxy-dehydroepiandrosterone, and in that time much information has been gained on its occurrence, metabolism, ontogeny, immunomodulatory activity, cell proliferation, cortisol control in local tissues and neuroactivity. Additional knowledge about this steroid may elucidate its role in obesity, neurodegenerative disturbances such as Alzheimer's disease, or psychiatric disorders such as schizophrenia or depression. This review aims to provide a comprehensive summary of the available literature on 7α-hydroxy-dehydroepiandrosterone.

Key words

Dehydroepiandrosterone • 7α-hydroxy-dehydroepiandrosterone • Neurosteroid • Occurrence • Immunomodulatory effects • CYP7B • 11β-hydroxysteroid dehydrogenase

Corresponding author

L. Stárka, Department of Steroid Hormones, Institute of Endocrinology, Národní 8, 11694 Prague 1, Czech Republic.
E-mail: lstarka@endo.cz

Early studies

7α-hydroxy-dehydroepiandrosterone (3β,7α-dihydroxy-androst-5-en-17-one; 7-OH-DHEA), known initially from the microbial transformation of dehydroepiandrosterone (DHEA), was first isolated from human material by Okada et al. (1959) in the urine of a patient with adrenal carcinoma. In 1961 we published a simple method of 7-hydrox-DHEA synthesis that yielded both α and β isomers with a prevalence of the α-epimer (Stárka and Syhora 1960, Stárka 1961). The crystalline compound obtained enabled us to perform the chromatographic isolation and identification of 7α-OH-DHEA, and in minor concentrations also of the 7β-isomer, in the urine (Stárka et al. 1962) and plasma (Stárka and Hampl 1964) of healthy men and women, as well as to study the hepatic (Stárka and Kůtová 1962) and extrahepatic (Šulcová and Stárka 1963, Stárka 1965) 7-hydroxylation of DHEA. 7-hydroxylation was found to be common in various organs of experimental animals (rats, frogs, horses), increasing in the order adrenals – muscle – heart – liver – lung – spleen (Šulcová and Stárka 1963). The ontogeny of 7-OH-DHEA was studied in the human embryo, chorion, amniotic epithelium and amnion (Šulcová et al. 1967, Šulcová et al. 1968, Šulcová et al. 1976, Šulcová et al. 1982), with 7-hydroxylation of DHEA found to be starting at the 7th week of gestation and a maximum occurring at the 22-23rd week. 7-hydroxylation in a rat liver homogenate (Stárka and Kutová 1962) and by hepatic microsomal fraction was described and characterized nearly simultaneously by several authors (Šulcová and Stárka 1968, Heinrichs and Collás 1968, Heinrichs et al. 1967).

The further metabolic transformation of 7-OH-DHEA was mainly studied in the liver, where depending on conditions the oxidation yielded 7-oxo-DHEA, 7α-hydroxy-androst-4-ene-3,17-dione and 7α-hydroxytestosterone, whereas incubation of 7-oxo-DHEA with rat liver slices led to the reduction of the 7-oxo-group under the formation of 7α- and 7β-hydroxy-derivatives at an approximate ratio of 1:1 (Hampl and Stárka 1967). We also studied the epimerization of 7α/β-hydroxy-DHEAs and of steroid allyl-alcohols in general (Hampl and Stárka...
Research was accelerated by the hypothesis that DHEA is a “hormone of youth” and that its metabolites could participate in this role (Baulieu 1996). Doostzadeh and Morfin 1966, Doostzadeh 1975). Later, the relationship of 7-OH-DHEA in plasma to the stage of mammary carcinoma was demonstrated (Skinner et al. 1980).

After the pioneering research on 7-OH-DHEA in the sixties, nearly one generation passed before major further discoveries were made showing the importance of this steroid. Research was accelerated by the hypothesis that DHEA is a „hormone of youth” and that its metabolites could participate in this role (Baulieu 1996).

**Enzyme system responsible for 7-hydroxylation of DHEA**

The 7-hydroxylation of dehydroepiandrosterone was later confirmed in various tissues (adrenals, testis, liver), including the brain (Akwa et al. 1992, Akwa et al. 1993, Doostzadeh and Morfin 1996, Doostzadeh et al. 1997, Rose et al. 1997, Morfin and Stárka 2001, Chalbot and Morfin 2005a, Chalbot and Morfin 2012) and adipose tissue (Khalil et al. 1993, Khalil et al. 1995). The metabolism of DHEA and related 7-hydroxylated derivatives in human liver S9 fractions (Chalbot and Morfin 2005b) and in specific regions of the brain was also described (Weil-Engerer et al. 2003, Li and Bigelow 2010).

The enzyme system responsible for the 7-hydroxylation of DHEA was characterized in more detail in the liver, brain and prostate (Tabei et al. 1975, Doostzadeh and Morfin 1966, Doostzadeh et al. 1997, Doostzadeh et al. 1998, Attal-Khémis et al. 1998b, Robinzon et al. 2004, Chalbot and Morfin 2005a, Chalbot and Morfin 2006, Kim et al. 2004, Trap et al. 2005, Martin et al. 2001). Different P450s were found to be involved in the 7α- and 7β-hydroxylation of DHEA, and that in addition to CYP7B1 7-hydroxylase (identical to cholesterol 7-hydroxylase), CYP7B2 also takes part in the 7-hydroxylation of DHEA. A comparison of these findings with those obtained with brain microsomes suggested that tissue-specific P450 species are responsible for the 7α- and 7β-hydroxylation of DHEA (Doostzadeh et al. 1998). Microsomes contained most of the activity, except for in the brain where mitochondrial activity was primary (Doostzadeh and Morfin 1996). The system responsible for the 7-hydroxylation of 5-ene-steroids was fully characterized (Stapleton et al. 1995, Rose et al. 1997, Rose et al. 2001). It was concluded that Cyp7b is a 7α-hydroxylase participating in the synthesis of the neurosteroids 7α-hydroxy-DHEA, and 7α-hydroxy-pregnenolone in brain. This system differs from cholesterol 7-hydroxylase, and genomic Southern analysis has suggested that a single gene corresponding to CYP7B1 (also known as hct-1) is present in the mouse, rat, and human. CYP7B1 is unusual in that, unlike all other CYPs described until now, the primary site of expression is in the brain. Findings suggest that nuclear factor-κB (NF-κB) and activator protein AP-1 are involved in the tumor necrosis factor-α (TNF-α) -enhanced formation of the dehydroepiandrosterone metabolite 7α-OH-DHEA (Dulos et al. 2005). The ontogeny of the 7-hydroxylation system was also mapped in the mouse embryo (Bean et al. 2001).

For the preparation of pure 7-OH-DHEA, the 7-hydroxylation of DHEA in Saccharomyces cerevisiae (Vico et al. 2002) and Mucor racemous (Li et al. 2005) were used, and it was proposed that this system may reflect the conservation of an early signaling pathway of non-enzymatic reactions (Lathé 2002).

**The effects of 7-OH-DHEA**

As could be expected from the fact that molecular oxygen is essential for enzymatic 7-hydroxylation, antioxidant activity was found for DHEA and 7-OH-DHEA (Pelissier et al. 2004). The latter steroid exerted its anti-oxidant effect earlier than DHEA and mainly in the liver. As DHEA was found to possess an anti-glucocorticoid activity, it was crucial to determine whether its 7-oxygenated metabolites also exert such an effect. The anti-glucocorticoid activity of 7-OH-DHEA was demonstrated e.g. on the viability of plaque forming cells of cultured murine spleen lymphocytes incubated with dexamethasone (Hampl et al. 2006). As for DHEA, no specific receptors were found for 7-OH-DHEA and no binding to the glucocorticoid receptors could be demonstrated (Stárka et al. 1998, Muller et al. 2004, Muller et al. 2006).
An important contribution to the question of the role of 7-OH-DHEA was made by Chalbot and Morfin (2006). First, they demonstrated that 7-hydroxylated steroids produced in human tonsils enhance the immune response to tetanus toxoid and Bordetella pertussis antigens (Lafaye et al. 1999), and that second, the dexamethasone-induced apoptosis of mouse thymocytes is prevented by native 7α-hydroxy-steroids (Chmielewski et al. 2000). A similar effect was observed in murine spleenocytes (Šterzl et al. 1999). Several authors (Morfin and Cournachy 1994, Morfin et al. 2000, Hampl et al. 1997, Hampl et al. 2001) published further proof that 7-hydroxylated steroids are involved in a process that may participate in the physiological regulation of the body's immune response. Immunomodulatory cytokines in seminal plasma correlated with the content of 7-OH-DHEA (Hampl et al. 2000a,b, Pohanka et al. 2002, Šterzl et al. 2003). In rats with colitis, anti-inflammatory effects and changes in prostaglandin patterns were produced even more intensively by 7-hydroxy-epiandrosteron, a metabolite of 7-OH-DHEA (Hennebert et al. 2007c). An anti-proliferative activity of 7-oxygenated-DHEA metabolites that is not induced by inhibiting G6PD (glucose-6-phosphate dehydrogenase) or HMGR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) activity alone was also observed (Yoshida et al. 2003).

7-OH-DHEA in the brain

Numerous authors have paid attention to the presence and role of 7-oxygenated dehydroepiandrosterone derivatives in the brain (for review see Morfin and Starka 2001).

7-hydroxylated derivatives of dehydroepiandrosterone were found in the human ventricular cerebrospinal fluid (Stárka et al. 2009, Kancheva et al. 2011) and were compared with serum levels (Kancheva et al. 2010) in women with hydrocephalus. In shunt cerebrospinal fluid, 7-OH-DHEA could be even used as a prognostic factor for the success of surgical therapy (Sosvorová et al. 2012, Sosvorová et al. 2015a,b).

Particular attention has been paid to the role of 7-OH-DHEA in the brain as a neuroactive steroid. The pioneer works in this field were reviewed by Morfin and Stárka (2001). DHEA enhances memory and immune function but has no known dedicated receptor; local metabolism may govern its activity (Rose et al. 2001, Stárka et al. 2015). There were several contributions to knowledge on the localization, production in various areas of the brain, the conditions for 7-hydroxylation and further metabolism and the effects as a neurosteroid of 7-OH-DHEA (Jellinck et al. 2001, Jellinck et al. 2005, Li and Bigelow 2010, Rose et al. 2001, Kazihnitková et al. 2004). In contrast to DHEA, 7-hydroxylated derivatives were shown to mediate neuroprotection (Jellinck et al. 2005, Chalbot and Morfin 2005a,b, Pringle et al. 2003, Yau et al. 2003, Yau et al. 2006).

Several very important findings were that the interconvertible 7-oxygenated Δ4-steroids, namely 7α-, 7β-hydroxy-DHEA and 7-oxo-DHEA, can be substrates for 11β-hydroxysteroid dehydrogenase type I (11β-HSD), and so 7-OH-DHEA and other 7-hydroxylated C19-steroids function as factors maintaining the balance of local cortisol and cortisone concentrations (Hennebert et al. 2007a,b,c, Hennebert et al. 2009, Muller et al. 2006). These important interconversions locally controlling glucocorticoid levels in various tissues were also confirmed by other authors (Robinzon et al. 2003). The balance between 7β-hydroxy- Δ4-C19 steroids and their 7α-hydroxy- counterparts is regulated by type I 11β-hydroxysteroid dehydrogenase (HSD11B1), which is capable (in addition to catalyzing the conversion of inactive cortisone to bioactive cortisol) of converting the 7α-hydroxy- Δ4-C19 steroids via 7-oxo-steroid to their 7β-hydroxy-counterparts. This view was supported by the findings (Steckelbroeck et al. 2002) of high levels of CYP7B1 mRNA in brain tissue as well in combination with the ubiquitous presence of 7α-hydroxylase activity in the human temporal lobe, which led to the assumption of a neuroprotective function of the enzyme such as regulation of the immune response or counteracting the deleterious effects of neurotoxic glucocorticoids, rather than a distinct brain specific function such as neurostimulation or neuromodulation. However, the role of these steroid transformations has been questioned, and it has been suggested that other as-yet unknown mechanisms responsible for the anti-glucocorticoid activity of DHEA and its metabolites may be found (Jellinck et al. 2001, Gottfried-Blackmore et al. 2013). Investigations of the metabolism of DHEA in E(t)C neuronal cells suggest that other alternate mechanisms than 11β-HSD must also be at play to explain the in vivo anti-glucocorticoid properties of DHEA and its 7-hydroxy-metabolites (Gottfried-Blackmore et al. 2013). 7-hydroxylated metabolites of DHEA might be responsible for some of the functions previously ascribed to estrogens in the brain (Jellinck et al. 2001).
Local control of the cortisol/cortisone ratio by 7-oxygeneratated DHEA metabolites was suggested as a possible factor in some neurodegenerative diseases such as Alzheimer’s dementia (Kim et al. 2003, Bičíková et al. 2004, Vaňková et al. 2016) and psychiatric disorders such as depression and anxiety (Dušková et al. 2015, Hill et al. 2016), schizophrenia (Bičíková et al. 2011) and premenstrual syndrome (Dušková et al. 2016). Decreased levels of DHEA were found in the cerebrospinal fluid of patients with Alzheimer’s disease (AD), whereas its 7-oxygenated metabolites were not significantly changed (Kim et al. 2003). Increased 7-OH-DHEA was found in the plasma of AD patients (Kim et al. 2003, Attal-Khémis et al. 1998a), whereas others found lower levels in serum (Bičíková et al. 2004, Vaňková et al. 2016). Changes in the ratio of 7α/7β-hydroxy-DHEA were seen in patients with dementia, and this ratio was sufficient for the differentiation between vascular and Alzheimer’s dementia (Kim et al. 2003).

Levels of 7-OH-DHEA were found to be lower in the plasma of patients with Alzheimer’s dementia (AD) than in controls, and even lower than in the plasma of patients with vascular dementia (Bičíková et al. 2004, Hampl and Bičíková 2010).

7-OH-DHEA has been measured in the individual brain regions of AD patients and aged non-demented controls. A significantly higher synthesis of 7α-hydroxy-DHEA in the frontal cortex was observed compared with that in other brain regions. In addition, a trend toward a significant negative correlation was found between the density of cortical amyloid deposits and the amount of 7α-hydroxy-DHEA formed in the frontal cortex (Weill-Engerer et al. 2003). Additionally, a reduced (50%) activity of 7-hydroxylating CYP7B system was found in the hippocampus of primates with AD (Yau et al. 2003).

Other effects of 7-OH-DHEA

Since one close metabolite of 7-OH-DHA is 7-oxo-DHEA (Marwah et al. 2002), which is claimed to possess some thermogenic activity as an ergosteroid (Lardy et al. 1995), it is possible that at least some of the effects of 7-OH-DHEA are actually exerted by its metabolites.

Another related steroid, 5-androstene-3β,7β,17β-triol, exhibits glucocorticoid-opposing and immune-modulating activity (Ahlem et al. 2011), and because its plasma levels positively correlate with BMI in healthy men and women, the authors suggested its compensatory role in preventing the development of metabolic syndrome (Auci et al. 2011). 5-androstene-3β,7β,17β-triol (β-AET), an active metabolite of dehydroepiandrosterone (DHEA), reversed the glucocorticoid induced suppression of IL-6, IL-8 and osteoprotegerin production (Malik et al. 2010). This steroid also influences estrogen receptor beta signaling (Pettersson et al. 2010).

Recently, attention has been given to various situations in which the levels of 7-OH-DHEA are different from control samples, as e.g. in the course of gravidity and following childbirth (Hill et al. 2010), during the female menstrual cycle in connection with changes of mood (Dušková et al. 2011), obesity (Sedláčková et al. 2012, Máčová et al. 2014), and during adrenal function testing by the ACTH or hypoglycemic tests (Dušková et al. 2016).

Methods for the analysis and production of 7-OH-DHEA

The first RIA of 7-OH-DHEA was described by Skinner et al. (1977). Lapčík later used this method to describe the course of plasma levels of men and women during their life spans, finding a remarkable decrease with age after 40 (Lapčík et al. 1998, Lapčík et al. 1999, Hampl et al. 2001). Presently, LC/MS or GC/MS methods are preferred (Hampl et al. 2002, Hill et al. 2001, Li et al. 2010, Sosvorová et al. 2015a,b, Matsuzaki et al. 2004).

Simplified chemical approaches leading to the production of 7α-7β-hydroxy-DHEA in quantities that made them readily available to researchers, and the production of isotope-labeled compounds, 3H-, 4H, and 14C-labeled 7α-7β-hydroxy-DHEA, were summarized by Feroud et al. (2012).

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The study was supported by MH CZ – DRO (Institute of Endocrinology – EU, 00023761) and by the MEYS CR (OP RDE, Excellent research – ENDO.CZ).
References

AHLEM CN, AUCI DL, NICOLETTI F, PIETERS R, KENNEDY MR, PAGE TM, READING CL, ENIOUTINA EY, FRINCKE JM: Pharmacology and immune modulating properties of 5-androstene-3β,7β,17β-triol, a DHEA metabolite in the human metabolome. *J Steroid Biochem Mol Biol* **126**: 87-94, 2011.

AKWA Y, MORFIN RF, ROBEL P, BAULIEU EE: Neurosteroid metabolism. 7α-Hydroxylation of dehydroepiandrosterone and pregnenolone by rat brain microsomes. *Biochem J* **288**: 959-964, 1992.

AKWA Y, SANANÉS N, GOÛZOU M, ROBEL P, BAULIEU EE, LE GOASCOGNE C: Astrocytes and neurosteroids: metabolism of pregnenolone and dehydroepiandrosterone. Regulation by cell density. *J Cell Biol* **121**: 135-143, 1993.

ATTAL-KHÉMIS S, DALMEYDA V, MICHOT JL, ROUDIER M, MORFIN R: Increased total 7α-hydroxy-dehydroepiandrosterone in serum of patients with Alzheimer's disease. *J Gerontol A Biol Sci Med Sci* **53**: B125-B132, 1998a.

ATTAL-KHÉMIS S, DALMEYDA V, MORFIN R: Change of 7α-hydroxy-dehydroepiandrosterone levels in serum of mice treated by cytochrome P450-modifying agents. *Life Sci* **63**: 1543-1553, 1998b.

AUCI DL, AHLEM CN, KENNEDY MR, PAGE TM, READING CL, FRINCKE JM: A potential role for 5-androstene-3β,7β,17β-triol in obesity and metabolic syndrome. *Obesity (Silver Spring)* **19**: 806-811, 2011.

BAULIEU EE: Dehydroepiandrosterone (DHEA): a fountain of youth? *J Clin Endocrinol Metab* **81**: 3147-3151, 1996.

BEAN R, SECKL JR, LATHE R, MARTIN C: Ontogeny of the neurosteroid enzyme Cyp7b in the mouse. *Mol Cell Endocrinol* **174**: 137-144, 2001.

BICÍKOVÁ M, ŘÍPOVÁ D, HILL M, JIRÁK R, HAVLÍKOVÁ H, TALLOVÁ J, HAMPL R: Plasma levels of 7-hydroxylated dehydroepiandrosterone (DHEA) metabolites and selected amino-thiols as discriminatory tools of Alzheimer's disease and vascular dementia. *Clin Chem Lab Med* **42**: 518-524, 2004.

BICÍKOVÁ M, HAMPL R, HILL M, ŘÍPOVA D, MOHR P, PUTZ Z: Neuro- and immunomodulatory steroids and other biochemical markers in drug-naive schizophrenia patients and the effect of treatment with atypical antipsychotics. *Neuro Endocrinol Lett* **32**: 141-147, 2011.

CEDARD L, FILLMANN B, KNUPPEN R, LISBOA BP, BREUER H: The metabolism and aromatization of 7-substituted C-19 steroids in the placenta. *Hoppe Seylers Z Physiol Chem* **338**: 89-99, 1964.

CHALBOT S, MORFIN R: Neurosteroids: metabolism in human intestine microsomes. *Steroids* **70**: 319-326, 2005a.

CHALBOT S, MORFIN R: Human liver S9 fractions: metabolism of dehydroepiandrosterone, epiandrosterone, and related 7-hydroxylated derivatives. *Drug Metab Dispos* **33**: 563-569, 2005b.

CHALBOT S, MORFIN R: Dehydroepiandrosterone metabolites and their interactions in humans. *Drug Metabol Drug Interact* **22**: 1-23, 2006.

CHALBOT S, MORFIN R: Cytochrome P450-7B1 and 11β-hydroxysteroid dehydrogenase type 1 distribution in human tissues. *Horm Mol Biol Clin Invest* **9**: 179-189, 2012.

CHMIELEWSKI V, DRUPT F, MORFIN R: Dexamethasone-induced apoptosis of mouse thymocytes: prevention by native 7alpha-hydroxysteroids. *Immunol Cell Biol* **78**: 238-246, 2000.

COUCH RA, SKINNER SJ, TOBLER CJ, DOOUSS TW: The in vitro synthesis of 7-hydroxy-dehydroepiandrosterone by human mammary tissues. *Steroids* **26**: 1-15, 1975.

DOOSTZADEH J, MORFIN R: Studies of the enzyme complex responsible for pregnenolone and dehydroepiandrosterone 7α-hydroxylation in mouse tissues. *Steroids* **61**: 613-620, 1996.

DOOSTZADEH J, COTILLON AC, MORFIN R: Dehydroepiandrosterone 7α- and 7β-hydroxylation in mouse brain microsomes. Effects of cytochrome P450 inhibitors and structure-specific inhibition by steroid hormones. *J Neuroendocrinol* **9**: 923-928, 1997.

DOOSTZADEH J, COTILLON AC, BENALYCHÉRIF A, MORFIN R: Inhibition studies of dehydroepiandrosterone 7α- and 7β-hydroxylation in mouse liver microsomes. *Steroids* **63**: 608-614, 1998.

DULOS J, KAPTEIN A, KAVELAARS A, HEIJNEN C, BOOTS A: Tumour necrosis factor-alpha stimulates dehydroepiandrosterone metabolism in human fibroblast-like synoviocytes: a role for nuclear factor-kappaB and activator protein-1 in the regulation of expression of cytochrome p450 enzyme 7b. *Arthritis Res Ther* **7**: R1271-R1280, 2005.
DUŠKOVÁ M, ŠIMŮNKOVÁ K, HILL M, STÁRKA L: 7-hydroxylated derivatives of dehydroepiandrosterone as possibly related to menstrual mood change in healthy women. Endocr Regul 45: 131-137, 2011.

DUŠKOVÁ M, HILL M, BIČÍKOVÁ M, ŠRÁMKOVÁ M, ŘÍPOVÁ D, MOHR P, STÁRKA L: Steroid metabolom in men with mood and anxiety disorders. Physiol Res 64 (Suppl 2): S275-S282, 2015.

DUŠKOVÁ M, SOSVOROVÁ L, HILL M, BIČÍKOVÁ M, ŠRÁMKOVÁ M, ŘÍPOVÁ D, MOHR P, STÁRKA L: The response of C19-Δ5-steroids to ACTH stimulation and hypoglycemia in insulin tolerance test for adrenal insufficiency. Prague Med Report 117: 98-107, 2016.

FAREDIN I, FAZEKAS AG, TÓTH I, KÓKAI K, JULESZ M: Transformation in vitro of [4-14-C]-dehydroepiandrosterone into 7-oxygenated derivatives by normal human male and female skin tissue. J Invest Dermatol 52: 357-361, 1969.

FERROUD C, REVIAL G, MORFIN R: Chemical and biochemical approaches to the production of 7-hydroxylated C19-steroids. Horm Mol Biol Clin Investig 10: 293-299, 2012.

GOTTFRIED-BLACKMORE A, JELLINCK PH, VECCHIARELLI HA, MASHEEB Z, KAUFMANN M, MCEWEN BS, BULLOCH K: 7α-hydroxylation of dehydroepiandrosterone does not interfere with the activation of glucocorticoids by 11β-hydroxysteroid dehydrogenase in E(t)C cerebellar neurons. J Steroid Biochem Mol Biol 138: 290-297, 2013.

HAMPL R, BIČÍKOVÁ M: Neuroimmunomodulatory steroids in Alzheimer dementia. J Steroid Biochem Mol Biol 119: 97-104, 2010.

HAMPL R, STÁRKA L: In vitro metabolic transformation of 7α-hydroxy-dehydroepiandrosterone in rat liver, adrenal and testis. Endocr Exper 1: 5-13, 1967.

HAMPL R, STÁRKA L: Epimerisation of naturally occurring C19-steroid allylic alcohols by rat liver preparations. J Steroid Biochem 1: 47-56, 1969.

HAMPL R, MORFIN R, STÁRKA L: 7-Hydroxylated C19-steroids: what are they good for? Endocr Regul 31: 211-218, 1997.

HAMPL R, LAČÍK O, HILL M, KLÁK J, KASAL A, NOVÁČEK A, ŠTERZL I, ŠTERZL J, STÁRKA L: 7-Hydroxy-dehydroepiandrosterone - a natural antiglucocorticoid and a candidate for steroid replacement therapy? Physiol Res 49 (Suppl 1): S107-S112, 2000a.

HAMPL R, HILL M, ŠTERZL I, STÁRKA L: Immunomodulatory 7-hydroxylated metabolites of dehydroepiandrosterone are present in human semen. J Steroid Biochem Mol Biol 75: 273-276, 2000b.

HAMPL R, HILL M, STÁRKA L: 7-Hydroxydehydroepiandrosterone epimers in the life span. J Steroid Biochem Mol Biol 78: 367-372, 2001.

HAMPL R, HILL M, STÁRKA L: Detection and quantification of 7-hydroxydehydroepiandrosterone epimers in three body fluids. Coll Czech Chem Commun 67: 10-18, 2002.

HEINRICHS WL, COLÁS A: The selective stimulation, inhibition, and physicochemical alteration of the 7- and 16α-hydroxylases of 3β-hydroxyandrost-5-en-17-one and drug-metabolizing enzymes in hepatic microsomal fractions. Biochemistry 7: 2273-2280, 1968.

HEINRICHS WL, MUSHEN RL, COLÁS A: The 7β-hydroxylation of 3β-hydroxyandrost-5-en-17-one by hepatic microsomes. Steroids 9: 23-40, 1967.

HENNEBERT O, CHALBOT S, ALRAN S, MORFIN R: Dehydroepiandrosterone 7α-hydroxylation in human tissues: possible interference with type I 11β-hydroxysteroid dehydrogenase-mediated processes. J Steroid Biochem Mol Biol 104: 326-333, 2007a.

HENNEBERT O, LE MÉE S, PERNELLE C, MORFIN R: 5α-androstane-3β,17β-triol and 5α-androstane-3β,7β,17β-triol as substrates for the human 11β-hydroxysteroid dehydrogenase type 1. Steroids 72: 855-864, 2007b.

HENNEBERT O, PERNELLE C, FERROUD C, MORFIN R: 7α- and 7β-hydroxy-epiandrosterone as substrates and inhibitors for the human 11β-hydroxysteroid dehydrogenase type 1. J Steroid Biochem Mol Biol 105: 159-165, 2007c.

HENNEBERT O, MONTES M, FAVRE-REGUILLON A, CHERMETTE H, FERROUD C, MORFIN R: Epimerase activity of the human 11β-hydroxysteroid dehydrogenase type 1 on 7-hydroxylated C19-steroids. J Steroid Biochem Mol Biol 114: 57-63, 2009.
HILL M, LAPČÍK O, HAVLÍKOVÁ, MORFIN R, HAMPL R: 7-Hydroxydehydroepiandrosterone epimers in human serum and saliva. Comparison of gas chromatography-mass spectrometry and radioimmunoassay. *J Chromatogr A* 935: 297-307, 2001.

HILL M, PAŘÍZEK A, KANCHEVA R, DUŠKOVÁ M, VELÍKOVÁ M, KŘÍŽ L, KLÍMKOVÁ M, PAŠKOVÁ A, ŽÍŽKA Z, MATUCHA P, MELOUN M, STÁRKA L: Steroid metabolome in plasma from the umbilical artery, umbilical vein, maternal cubital vein and in amniotic fluid in normal and preterm labor. *J Steroid Biochem Mol Biol* 121: 594-610, 2010.

HILL M, ŘÍPOVÁ D, MOHR P, KRATOCHVÍLOVÁ Z, VELIKOVÁ M, BÍČÍKOVÁ M, DUŠKOVÁ M, STÁRKA L: Circulating C19 steroids and progesterone metabolites in women with acute depression and anxiety disorders. *Horm Mol Biol Clin Investig* 26: 153-164, 2016.

JANATA J, JANATOVÁ V, STÁRKA L: L aromatisation of 7α-hydroxydehydroepiandrosterone and other androgens by ovarian and placental tissue culture. *Akad Wiss(Berlin) Abh, Klasse Medizin* 3: 783-786, 1965.

JELLINCK PH, LEE SJ, M CEWEN BS: Metabolism of dehydroepiandrosterone by rat hippocampal cells in culture: possible role of aromatization and 7-hydroxylation in neuroprotection. *J Steroid Biochem Mol Biol* 78: 313-317, 2001.

JELLINCK PH, CROFT G, MCEWEN BS, GOTTTFRIED-BLACKMORE A, JONES G, BYFORD V, BULLOCH K: Metabolism of dehydroepiandrosterone by rodent brain cell lines: relationship between 7-hydroxylation and aromatization. *J Steroid Biochem Mol Biol* 93: 81-86, 2005.

KANCHEVA R, HILL M, NOVÁK Z, CHRASTINA J, VELIKOVÁ M, KANCHEVA L, RIHA I, STÁRKA L: Peripheral neuroactive steroids may be as good as the steroids in the cerebrospinal fluid for the diagnostics of CNS disturbances. *J Steroid Biochem Mol Biol* 119: 35-44, 2010.

KANCHEVA R, HILL M, NOVÁK Z, CHRASTINA J, KANCHEVA L, STÁRKA L: Neuroactive steroids in periphery and cerebrospinal fluid. *Neuroscience* 191: 22-27, 2011.

KAZHNITKOVÁ H, TEJKALOVÁ H, BENESOVÁ O, BÍČÍKOVÁ M, HILL M, HAMPL R: Simultaneous determination of dehydroepiandrosterone, its 7-hydroxylated metabolites, and their sulfates in rat brain tissues. *Steroids* 69: 667-674, 2004.

KHALIL MW, STRUTT B, VACHON D, KILLINGER DW: Metabolism of dehydroepiandrosterone by cultured human adipose stromal cells: identification of 7α-hydroxydehydroepiandrosterone as a major metabolite using high performance liquid chromatography and mass spectrometry. *J Steroid Biochem Mol Biol* 46: 585-595, 1993.

KHALIL MW, STRUTT B, KILLINGER DW: 7α-Hydroxylation of the adrenal androgens dehydroepiandrosterone and androst-5-ene-3β,17β-diol predominates in differentiating human adipose stromal cells. *Ann N Y Acad Sci* 774: 316-318, 1995.

KIM SB, HILL M, KWAK YT, HAMPL R, JO DH, MORFIN R: Neurosteroids: Cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics. *J Clin Endocrinol Metab* 88: 5199-5206, 2003.

KIM SB, CHALBOT S, POMPON D, JO DH, MORFIN R: The human cytochrome P4507B1: catalytic activity studies. *J Steroid Biochem Mol Biol* 92: 383-389, 2004.

LAFAYE P, CHMIELEWSKI V, NATO F, MAZIÉ JC, MORFIN R: The 7α-hydroxysteroids produced in human tonsils enhance the immune response to tetanus toxoid and Bordetella pertussis antigens. *Biochim Biophys Acta* 1472: 222-231, 1999.

LAPČÍK O, HAMPL R, HILL M, BÍČÍKOVÁ M, STÁRKA L: Immunoassay of 7-hydroxysteroids: 1. Radioimmuno assay of 7beta-hydroxydehydroepiandrosterone. *J Steroid Biochem Mol Biol* 67: 439-445, 1998.

LAPČÍK O, HAMPL R, HILL M, STÁRKA L: Immunoassay of 7-hydroxysteroids: 2. Radio- immunoassay of 7α-hydroxy-dehydroepiandrosterone. *J Steroid Biochem Mol Biol* 71: 231-237, 1999.

LARDY H, PARTRIDGE B, KNEER N, WEI Y: Ergosteroids: induction of thermogenic enzymes in liver of rats treated with steroids derived from dehydroepiandrosterone. *Proc Natl Acad Sci USA* 92: 6617-6619, 1995.

LATHE R: Steroid and sterol 7-hydroxylation: ancient pathways. *Steroids* 67: 967-977, 2002.

LI A, BIGELOW JC: The 7-hydroxylation of dehydroepiandrosterone in rat brain. *Steroids* 75: 404-410, 2010.
LI A, MAY MP, BIGELOW JC: An LC/MS method for the quantitative determination of 7α-OH DHEA and 7β-OH DHEA: an application for the study of the metabolism of DHEA in rat brain. Biomed Chromatogr 24: 833-837, 2010.

LI H, LIU HM, GE W, HUANG L, SHAN L: Synthesis of 7α-hydroxy-dehydroepiandrosterone and 7β-hydroxy-dehydroepiandrosterone. Steroids 70: 970-973, 2005.

MÁČOVÁ L, BÍČÍKOVÁ M, ZAMRAZILOVÁ H, HILL M, KAZIHNIKOVÁ H, SEDLÁČKOVÁ B, STÁRKA L: Reduced levels of circulating 7α-hydroxy-dehydroepiandrosterone in treated adolescent obese patients. Physiol Res 63: 95-101, 2014.

MALIK AK, KHALDOYANIDI S, AUCI DL, MILLER SC, AHLEM CN, READING CL, PAGE T, FRINCKE JM: 5-Androstene-3β,7β,17β-triol (β-AET) slows thermal injury induced osteopenia in mice: relation to aging and osteoporosis. PLoS One 5: e13566, 2010.

MARTIN C, BEAN R, ROSE K, HABIB F, SECKL J: cyp7b1 catalyses the 7α-hydroxylation of dehydroepiandrosterone and 25-hydroxycholesterol in rat prostate. Biochem J 355: 509-515, 2001.

MORFIN R, COURCEAY G: Pregnenolone and dehydroepiandrosterone as precursors of native 7-hydroxylated metabolites which increase the immune response in mice. J Steroid Biochem Mol Biol 50: 91-100, 1994.

MORFIN R, STÁRKA L: Neurosteroid 7-hydroxylation products in the brain. Int Rev Neurobiol 46: 79-95, 2001.

MORFIN R, LAFAYE P, COTILLON AC, NATO F, CHMIELEWSKI V, POMPON D: 7α-hydroxy-dehydroepiandrosterone and immune response. Ann N Y Acad Sci 917: 971-982, 2000.

MULLER C, CLUZEAUD F, PINON GM, RAFESTIN-OBLIN ME, MORFIN R: Dehydroepiandrosterone and its 7-hydroxylated metabolites do not interfere with the transactivation and cellular trafficking of the glucocorticoid receptor. J Steroid Biochem Mol Biol 92: 469-476, 2004.

MULLER C, HENNEBERT O, MORFIN R: The native anti-glucocorticoid paradigm. J Steroid Biochem Mol Biol 100: 95-105, 2006.

OKADA M, FUKUSHIMA DK, GALLAGHER TF: Isolation and characterization of 3β-hydroxy-Δ5-steroids in adrenal carcinoma. J Biol Chem 234: 1688-1692, 1959.

PELISSIER MA, TRAP C, MALEWIAK MI, MORFIN R: Antioxidant effects of dehydroepiandrosterone and 7α-hydroxy-dehydroepiandrosterone in the rat colon, intestine and liver. Steroids 69: 137-144, 2004.

PETTERSSON H, LUNDQVIST J, NORLIN M: Effects of CYP7B1-mediated catalysis on estrogen receptor activation. Biochim Biophys Acta 1801: 1090-1097, 2010.

POHANKA M, HAMPL R, ŠTERZL I, STÁRKA L: Steroid hormones in human semen with particular respect to dehydroepiandrosterone and its immunomodulatory metabolites. Endocr Regul 36: 79-86, 2002.

PRINGLE AK, SCHMIDT W, DEANS JK, WULFERT E, REYMANN KG, SUNDSTROM LE: 7-Hydroxylated epiandrosterone (7-OH-EPIA) reduces ischaemia-induced neuronal damage both in vivo and in vitro. Eur J Neurosci 18: 117-124, 2003.

ROBINZON B, MICHAEL KK, RIPP SL, WINTERS SJ, PROUGH RA: Glucocorticoids inhibit interconversion of 7-hydroxy and 7-oxo metabolites of dehydroepiandrosterone: a role for 11β-hydroxysteroid dehydrogenases? Arch Biochem Biophys 412: 251-258, 2003.

ROBINZON B, MILLER KK, PROUGH RA: Biosynthesis of [3H]7α-hydroxy-, 7 β-hydroxy-, and 7-oxo-dehydroepiandrosterone using pig liver microsomal fractions. Anal Biochem 333: 128-135, 2004.

ROSE K, ALLAN A, GAULDIE S, STAPLETON G, DOBBIE L, DOTT K, MARTIN C, WANG L, HEDLUND E, SECKL JR, GUSTAFSSON JA, LATHE R: Neurosteroid hydroxylase CYP7B: vivid reporter activity in dentate gyrus of gene-targeted mice and abolition of a widespread pathway of steroid and oxysterol hydroxylation. J Biol Chem 276: 23937-23944, 2001.
ROSE KA, STAPLETON G, DOTT K, KIENY MP, BEST R, SCHWARZ M, RUSSELL DW, BJÖRKHEM I, SECKL J, LATHE R: Cyp7b, a novel brain cytochrome P450, catalyzes the synthesis of neurosteroids 7α-hydroxy dehydroepiandrosterone and 7α-hydroxy pregnenolone. Proc Natl Acad Sci U S A 94: 4925-4930, 1997.

SEDLÁČKOVÁ B, DUŠÁTKOVÁ L, ZAMRAZILOVÁ H, MATUCHA P, BÍČÍKOVÁ M, STÁRKA L: 7-oxygenated derivatives of dehydroepiandrosterone and obesity. Prague Med Report 113: 147-155, 2012.

SKINNER SJ, TOBLER CJ, COUCH RA: A radioimmunoassay for 7α-hydroxy-dehydroepiandrosterone in human plasma. Steroids 30: 315-330, 1977.

SKINNER SJ, COUCH RA, THAMBYAH S, DOBBS RJ, JORDAN SM, MASON B, KAY RG: 7α-hydroxydehydroepiandrosterone and adrenogenital syndrome in breast cancer patients. Eur J Cancer 16: 223-228, 1980.

STAPLETON G, STEEL M, RICHARDSON M, MASON JO, ROSE KA, MORRIS RG, LATHE R: A novel cytochrome P450 expressed primarily in brain. J Biol Chem 270: 29739-29745, 1995.
ŠTERZL I, HAMPL R, ŠTERZL J, VOTRUBA J, STÁRKA L: 7β-OH-DHEA counteracts dexamethasone induced suppression of primary immune response in murine spleenocytes. *J Steroid Biochem Mol Biol* **71**: 133-137, 1999.

ŠTERZL I, HAMPL R, HILL M, HRDÁ P, MATUCHA P: Immunomodulatory cytokines in human seminal plasma correlate with immunomodulatory steroids. *Steroids* **68**: 725-731, 2003.

ŠULCOVÁ J, STÁRKA L: Extrahepatic 7alpha-hydroxylation of dehydroepiandrosterone. *Experientia* **19**: 1-4, 1963.

ŠULCOVÁ J, STÁRKA L: Characterisation of microsomal dehydroepiandrosterone 7-hydroxylase from rat liver. *Steroids* **12**: 113-126, 1968.

ŠULCOVÁ J, STÁRKA L, JIRÁSEK JE: 7α-Hydroxylation of dehydroepiandrosterone by the steroidogenic tissues and the liver of human embryo in the first trimester of pregnancy in vitro. *Gen Comp Endocr* **9**: 497, 1967.

ŠULCOVÁ J, ČAPKOVÁ A, JIRÁSEK JE, STÁRKA L: 7-hydroxylation of dehydroepiandrosterone in human foetal liver, adrenals and chorion in vitro. *Acta Endocrinol (Copenh)* **59**: 1-9, 1968.

ŠULCOVÁ J, JIRÁSEK JE, CARLSTEDT-DUKE J, STÁRKA L: 7-Hydroxylation of dehydroepiandrosterone in human amniotic epithelium. *J Steroid Biochem* **7**: 101-104, 1976.

ŠULCOVÁ J, STÁRKA L, JIRÁSEK JE: Metabolism of C19-delta-5-3β-hydroxysteroids in the term human amnion. *Endocrinol Exp* **16**: 9-17, 1982.

Tabei T, Fukushima K, Heinrichs WL: Enzymatic oxidation and reduction of C19-delta5-3beta-hydroxysteroids by hepatic microsomes. IV. Induction of DHEA hydroxylases and aminopyrine N-demethylase in immature male rats by androgens. *Endocrinology* **96**: 815-819, 1975.

TRAP C, NATO F, Chalbot S, Kim SB, Lafaye P, Morfin R: Immunohistochemical detection of the human cytochrome P450B1: production of a monoclonal antibody after cDNA immunization. *J Neuroimmunol* **159**: 41-47, 2005.

Vaňková M, Hill M, Rusina R, Vaňková H, Včelák J, Vacínová G, Dvořáková K, Lukášová P, Vejražková D, Velíková M, Jarolimová E, Holmerová I, Bendlová B, Stárka L: Circulating steroids as predictors of Alzheimer's disease. *J Steroid Biochem Mol Biol* **158**: 157-177, 2016.

Vico P, Cauet G, Rose K, Lathe R, Degryse E: Dehydroepiandrosterone (DHEA) metabolism in Saccharomyces cerevisiae expressing mammalian steroid hydroxylase CYP7B: Ayr1p and Fox2p display 17β-hydroxysteroid dehydrogenase activity. *Yeast* **19**: 873-886, 2002.

Weill-Engerer S, David JP, Saizovitch V, Liere P, Schumacher M, Delacourte A, Bauleiu EE, Akwa Y: In vitro metabolism of dehydroepiandrosterone (DHEA) to 7α-hydroxy-DHEA and Delta5-androstene-3β,17β-diol in specific regions of the aging brain from Alzheimer's and non-demented patients. *Brain Res* **969**: 117-125, 2003.

Yau JL, Rasmussen S, Andrew R, Graham M, Noble J, Olsson T, Fuchs E, Lathe R, Seckl JR: Dehydroepiandrosterone 7-hydroxylase CYP7B: predominant expression in primate hippocampus and reduced expression in Alzheimer's disease. *Neuroscience* **121**: 307-314, 2003.

Yau JL, Noble J, Graham M, Seckl JR: Central administration of a cytochrome P450-7B product 7α-hydroxypregnenolone improves spatial memory retention in cognitively impaired aged rats. *J Neurosci* **26**: 11034-11040, 2006.

Yoshida S, Honda A, Matsuzaki Y, Fukushima S, Tanaka N, Takagiwa A, Fujimoto Y, Miyazaki H, Salen G: Anti-proliferative action of endogenous dehydroepiandrosterone metabolites on human cancer cell lines. *Steroids* **68**: 73-83, 2003.