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Reduced Sulfotransferase SULT2A1 Activity in Patients With Alzheimer´s Disease

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Summary

Steroids are important components in the pathophysiology of Alzheimer's disease (AD). Although their role has been studied, the corresponding metabolomic data is limited. In the present study we evaluate the role of steroid sulfotransferase SULT2A1 in the pathophysiology of AD on the basis of circulating steroids (measured by GC-MS), in which the sulfation catalyzed by SULT2A1 dominates over glucuronidation (pregnenolone/sulfate, DHEA/sulfate, androstenediol/sulfate and 5α-reduced pregnane and androstane catabolites). To estimate a general trend of SUL2A1 activity in AD patients we compared the ratios of steroid conjugates to their unconjugated counterparts (C/U) in controls (11 men and 22 women) and AD patients (18 men and 16 women) for individual circulating steroids after adjustment for age and BMI using ANCOVA model including the factors AD status and gender. Decreased C/U ratio for the C19 steroids demonstrate an association between attenuated sulfation of C19 steroids in adrenal *zona reticularis* and the pathophysiology of AD.

Key words

Alzheimer's disease • SULT2A1 • GC-MS • Steroids

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Introduction

Alzheimer's disease (AD) represents more than one half of total dementias in seniors. The number of people living with dementia worldwide today is estimated at 44 million, set to almost double by 2030 and more than triple by 2050 (Prince *et al.* 2014).

AD is a neurodegenerative disease with progressive decline of episodic memory and impairment in other cognitive domains leading to dementia with loss of autonomy. Definite diagnosis of AD requires neuropathological confirmation from autopsy. Current diagnostic criteria for AD are mainly based on clinical and neuropsychological assessment (McKhann *et al.* 2011).

There is increasing evidence that pathological processes in the brain of AD patients may begin even 10-20 years before the first symptoms appear (Holtzman *et al.* 2011), therefore, the need for early diagnosis is important and other biomarkers may be useful.

Various factors including altered steroid biosynthesis may participate in the pathophysiology of AD. Steroid hormones are effective regulators of many physiological processes, including those in the brain (Hampl and Bicikova 2010). Steroid hormones active in neural tissue are neuroactive steroids, which arise in the gonads (in women only in the reproductive period) and in the adrenal glands (the main source of postmenopausal women) and into the central nervous system are

transported across the blood-brain barrier directly or in the form of precursors. However, a small portion of steroids, called neurosteroids, are produced directly in nervous tissue. The peripheral steroidogenesis may substantially influence neuronal activity in the brain.

Age-related deficit of sex steroid hormones increases the risk of dysfunction in hormone-responsive tissues, including brain. Age-dependent hormone depletion in the brain may result in diminished neuroprotection, which increases a risk of neurodegenerative diseases such as AD (Rosario and Pike 2008). AD is more prevalent in women, which may be explained by differences in life expectancy in women compared to men. However, incidence of AD is also higher in women (Ruitenberg *et al.* 2001). Nevertheless, the basis of higher vulnerability for females to AD remains unclear.

From the menopause, dehydroepiandrosterone (DHEA) remains the exclusive and adrenal tissue-specific source of sex steroids in women while in (65-75 year-old) men, the contribution of adrenal DHEA to the total androgens is approximately 40 % (Labrie 2010). Sex steroid hormones derived from DHEA show a similar decline from the age of 30 years in both genders, but there is a continuous supply of testosterone, estrone and estradiol from testes in men. The ovarian estradiol secretion in women ends at the menopause.

Age-related loss of sex steroid hormones is associated with numerous diseases such as tissue atrophy, bone loss, fat accumulation, type 2 diabetes mellitus, and also with cognition problems, memory loss and perhaps with AD (Labrie 2007). Alterations in steroid biosynthesis may contribute to the pathology of AD (Liu *et al.* 2013, Napolitano *et al.* 2014, Schumacher *et al.* 2014, Winkler and Fox 2013).

The endogenous sex steroids, estrogens, androgens, and progesterone, have effects on the brain, especially in perimenopausal and postmenopausal women (Yaffe *et al.* 2000). DHEA, which may be converted to estrogens locally in the brain, have been shown to enhance memory and learning functions (Vallee *et al.* 2001). Similarly, DHEA sulfate (DHEAS) influences brain function and affects memory capacity (Hunt *et al.* 2000). DHEA acting in the brain is either of peripheral origin, or may be synthesized directly in the brain and may be also converted into estrogens or androgens in brain tissues. Various brain areas express nuclear receptors for estrogens, androgens and progesterone. Besides the nuclear receptors, both androstane and pregnane steroids may also modulate the permeability of various ion channels such as excitatory glutamate receptors on one hand or inhibitory gamma-aminobutyric acid (GABA) receptors on the other hand (Bergeron *et al.* 1996, Majewska *et al.* 1990, Hill *et al.* 2015, Starka *et al.* 2015).

Steroid sulfates having distinct physiological role compared with their unconjugated counterparts are important precursors of further bioactive steroids (Strott 2002). The circulating levels of conjugated steroids are usually much higher than in their non-conjugated forms. The steroid conjugates are frequently metabolic end-products with excellent water solubility enabling their uncomplicated excretion into the urine. The conjugation generally alters biological activity (Strott 1996) and may even convert the unconjugated steroids to their biological antagonists (Park-Chung *et al.* 1997, 1999). Even though the sulfotransferase activity in primate brain is low (Kriz *et al.* 2005), the sulfation of brain neurosteroids might participate on the maintenance of brain function. Steroid sulfates are bound to serum proteins such as albumin, corticosteroid binding globulin (CBG), and sex hormone binding globulin (SHBG), and serve as circulating reservoir, while non-conjugated forms are more active at the tissue level.

Human SULT2A1 (sulfotransferase family 2A, dehydroepiandrosterone-preferring, member 1) is enzyme strongly expressed in fetal zone of the fetal adrenal gland (Barker *et al.* 1994), and in the *zona reticularis* of the mature adrenal gland. Besides the primary source of steroid sulfotransferase activity in adrenal *zona reticularis*, also the kidneys and small intestine exhibit significant sulfotransferase activities (Barker *et al.* 1994, Otterness *et al.* 1992). SULT2A1 expression is also detectable in ovary and prostate, in stomach and colon (Javitt *et al.* 2001), but these tissues produce the enzyme at a lower levels (Shimada *et al.* 2001). Besides the DHEA, the SULT2A1 catalyzes the sulfation of further androgens, their 5α/β-reduced catabolites, pregnenolone, 5α/β-reduced pregnanolone isomers and pregnanediols, and also the bile acids. Whereas the lower ratio of circulating sulfates to unconjugated form of steroids may indicate the lower overall SULT2A1 activity, we attempted to investigate whether the reduced ratios of steroid sulfates to their unconjugated counterparts are consistently lower in the AD and if so, whether these ratios may be used as markers of AD.

Materials and Methods

Subjects

A total of 34 patients with AD (16 women and 18 men) fulfilling NINCDS-ADRDA criteria (National Institute of Neurological Communicative Disorders and Stroke – Alzheimer's disease and Related Disorders Association) for probable Alzheimer´s disease and 33 controls of similar age as the patients (22 women and 11 men) participated in the study (Table 1).

Diagnosis of AD was confirmed by neuropsychological tests (Repeatable Battery for the Assessment of Neuropsychological Status, Montreal Cognitive Assessment, and Geriatric Depression Scale), biochemical examination of cerebrospinal fluid (β-amyloid peptides, total and phosphorylated τ-proteins) and magnetic resonance imaging of the brain. In our cohort of patients, 25 patients had AD, 3 patients had possible dementia with Lewy bodies in comorbidity and 6 patients had AD and significant subcortical ischemic white matter lesions consistent with the diagnosis of mixed dementia. Controls were examined with the same neuropsychological battery and had magnetic resonance imaging of the brain but lumbar puncture was not performed in these subjects.

Table 1. Comparison of anthropometric and biochemical characteristics in controls and in patients with Alzheimer´s disease (AD).

Variable	Mean (95% confidence limits)		ANOVA
	Controls	AD patients	*p*
Women	**n = 22**	**n = 16**	
Age (years)	66.9 (65.1, 68.8)	74.8 (72.1, 77.7)	**0.001**
BMI (kg/m^2)	24.9 (23.6, 26.4)	27.7 (25.8, 29.8)	0.123
WHR	0.81 (0.79, 0.83)	0.84 (0.82, 0.87)	0.172
BAI	32.3 (30.7, 34.1)	31.7 (29.8, 33.7)	0.758
HbA1c (%)	39.3 (37.9, 40.7)	35.2 (33.4, 36.9)	**0.016**
Total cholesterol (mmol/l)	5.03 (4.77, 5.31)	5.55 (5.19, 5.97)	0.134
HDL-cholesterol (mmol/l)	1.53 (1.4, 1.66)	1.75 (1.58, 1.94)	0.179
LDL-cholesterol (mmol/l)	2.89 (2.6, 3.19)	3.25 (2.9, 3.63)	0.295
Triacylglycerols (mmol/l)	1.14 (1.01, 1.3)	1.08 (0.945, 1.26)	0.743
Men	**n = 11**	**n = 18**	
Age (years)	68.8 (64.5, 73.1)	72.5 (69.2, 75.8)	0.327
BMI (kg/m^2)	27.2 (25.7, 28.8)	26.1 (25, 27.2)	0.393
WHR	0.95 (0.92, 0.99)	0.93 (0.91, 0.96)	0.457
BAI	26.2 (25.1, 27.3)	26.3 (25.4, 27.2)	0.914
HbA1c (%)	38 (36.3, 39.6)	38.1 (36.8, 39.5)	0.904
Total cholesterol (mmol/l)	4.58 (4.13, 5.11)	4.55 (4.2, 4.95)	0.94
HDL-cholesterol (mmol/l)	1.42 (1.32, 1.52)	1.31 (1.23, 1.39)	0.24
LDL-cholesterol (mmol/l)	2.57 (2.21, 3.02)	2.68 (2.37, 3.04)	0.771
Triacylglycerols (mmol/l)	1.11 (0.911, 1.34)	1.17 (1, 1.36)	0.756

The study subjects, patients as well as controls, did not use any drug known to interfere with the steroid biosynthesis and catabolism, especially corticoids, selective serotonin reuptake inhibitors, estrogens, hormone replacement therapy and nonsteroidal anti-inflammatory drugs.

Participants were examined after signing an informed consent approved by the Ethics Committee of the Institute of Endocrinology. For the evaluation of basic biochemical parameters and steroid metabolome, 10 ml of blood was withdrawn on fasting in the morning. Blood samples were centrifuged and stored at –80 °C until analyzed.

Anthropometric measurements

The waist to hip ratio (WHR) and body mass

index (BMI) were calculated. Body adiposity index (BAI), a surrogate measure of body fat, was calculated as described elsewhere (Freedman *et al.* 2012).

Analytical methods

The circulating levels of steroids and their polar conjugates were simultaneously measured using the previously described gas chromatography-mass spectrometry (GC-MS) method (Hill *et al.* 2010). In brief, the 1 ml of serum was extracted by 3 ml of diethyl-ether. The dry residue from the organic layer was partitioned between 1 ml of pentane and 1 ml of 80 % methanol-water mixture and the dry residue from the methanol-water layer was derivatized by 2 % of methoxylamine-hydrochloride in pyridine and then silylated by N,O-Bis(trimethylsilyl)trifluoroacetamide with 1 % of trimethylchlorosilane. The polar layer from the diethyl-ether extraction was hydrolyzed using the method by Dehennin *et al.* (1996) and then processed like the unconjugated steroids. Steroid polar conjugates contain mainly sulfates and glucuronides. In this study, we focused on the steroids in which the sulfates dominate over glucuronides such as Δ^5 steroids (adrenal gland pathway) and 5α-reduced C21 and C19 steroid metabolites.

Triacylglycerols, total cholesterol, and HDL-cholesterol were assayed by enzymatic colorimetric test, glycated hemoglobin A1c (HbA1c) was assayed by turbidimetric inhibition method (Roche, Cobas 6000). LDL-cholesterol was calculated as total cholesterol minus (triacylglycerols divided by 2.2) minus HDL-cholesterol.

Statistical analysis

Respecting the skewed distribution and non-constant variance in most dependent variables, these were transformed by power transformations to data symmetry and homoscedasticity prior further processing (Meloun *et al.* 2000). The homogeneity and distribution of the transformed data was checked by residual analysis as described elsewhere (Meloun *et al.* 2002).

To correctly explain the relationships between steroids, than product to precursor ratio (sulfates to unconjugated steroids ratio) and presence of AD for both genders, we used age- and BMI-adjusted ANCOVA with factors AD and gender. Anthropometric characteristics were evaluated using one-way ANOVA. Statistical software Statgraphics Centurion, version XV from Statpoint Inc. (Herndon, Virginia, USA) was used for ANOVA and ANCOVA testing.

Results

The differences between the ratios of conjugated to unconjugated steroids (C/U) in AD patients and controls with respect to gender are shown in Table 2.

As illustrated in Table 2, the C/U of the C21 steroids do not exhibit significant alterations in AD patients. On the other hand, the C/U of the Δ^5 C19 steroid DHEA and its 5α-reduced metabolites with a dominance of sulfates over the glucuronides (androsterone, epiandrosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol) are consistently lower in AD patients. Some C/U are significantly higher in men (pregnenolone, isopregnanolone, DHEA, epiandrosterone, 5α-androstane-3α,17β-diol) and the C/U in the androsterone negatively correlate with age. BMI do not significantly influence the values of C/U.

Discussion

Steroids are recognized as important components in the pathophysiology of AD. Although their role has been widely studied, the corresponding metabolomic data are limited (Maggio *et al.* 2015). To our knowledge, this study is the first attempt to evaluate the role of steroid sulfotransferase SULT2A1 in the pathophysiology of AD on the basis of steroid metabolome in the circulation. For this purpose we have selected the unconjugated steroids and their conjugated counterparts in which the sulfation catalyzed by SULT2A1 dominates over glucuronidation. Therefore we omit the 5β-reduced steroids, in which the glucuronidation is comparable with sulfation (Brochu and Belanger 1987). Furthermore, estrogens, which are extensively sulfated by estrogen preferring sulfotransferase SULT1E1, are also omitted (Tibbs *et al.* 2015). To estimate a general trend of SULT2A1 activity in AD patients, we have compared the ratios of steroid conjugates to their unconjugated counterparts (C/U) in controls and AD patients for individual circulating steroids after adjustment for age and BMI using the ANCOVA model.

Our results showing decreased C/U in the C19 steroids demonstrate at least an association between attenuated sulfation of C19 steroids in adrenal *zona reticularis* and the pathophysiology of AD. These data are compatible with the concept of "older" *zona reticularis* in AD patients compared with controls. Numerous studies demonstrate decline in adrenal androgen production with advancing age and an

Table 2. Ratios of conjugated steroids to their unconjugated counterparts in the circulation in patients with Alzheimer´s disease (AD) and in controls.

Conjugated to unconjugated steroids (C/U)			Mean (95 % confidence limits)		ANCOVA (p-values)			
			Women Controls: n=22 AD: n=16	Men Controls: n=11 AD: n=18	AD	Gender	Age	BMI
C21 steroids	*Pregnenolone sulfate/ pregnenolone*	Controls AD	51 (43, 59) 46 (38, 55)	73 (60, 89) 67 (57, 77)	0.451	**0.003**	0.574	0.054
	Allopregnanolone sulfate/ allopregnanolone	Controls AD	24 (21, 28) 25 (21, 30)	27 (22, 33) 34 (29, 41)	0.285	0.089	0.830	0.764
	Isopregnanolone sulfate/ isopregnanolone	Controls AD	42 (36, 49) 40 (33, 48)	69 (58,82) 54 (47,63)	0.219	**0.001**	0.364	0.418
C19 steroids	*DHEAS/ DHEA*	Controls AD	121 (102, 143) 81 (65, 100)	205 (165, 253) 169 (142, 200)	**0.046**	**<0.001**	0.735	0.361
	Androstenediol sulfate/ androstenediol	Controls AD	455 (358, 589) 301 (230, 400)	507 (363, 734) 389 (305, 505)	0.109	0.347	0.409	0.122
	Androsterone sulfate/ androsterone	Controls AD	1894 (1561, 2268) 1162 (876, 1498)	1398 (1031, 1834) 1053 (808, 1336)	**0.037**	0.227	**0.005**	0.501
	Epiandrosterone sulfate/ epiandrosterone	Controls AD	374 (312, 443) 211 (160, 269)	528 (419, 654) 426 (354, 505)	**0.016**	**<0.001**	0.103	0.982
	5α-Androstane-3α,17β-diol sulfate/ 5α-androstane-3α,17β-diol	Controls AD	301 (231, 391) 168 (118, 236)	567 (400, 801) 274 (206, 363)	**0.006**	**0.011**	0.329	0.573
	5α-Androstane-3β,17β-diol sulfate/ 5α-androstane-3β,17β-diol	Controls AD	1100 (874, 1357) 834 (611, 1098)	1611 (1210, 2085) 998 (772, 1258)	**0.048**	0.114	0.067	0.071

Evaluated by ANCOVA with Age and BMI as covariates and AD diagnosis and Gender as factors.

analogous diminution in the area represented by the *zona reticularis*, which is regulated by extra-adrenal modulators such as corticoliberin, adrenocorticotropic hormone, insulin, and transforming growth factor β (Auchus 2004, Dharia and Parker 2004, Ibanez *et al.* 1999, Sulcova *et al.* 1997). Although the DHEA is unlikely to have influence on the cognitive abilities in AD patients (Maggio *et al.* 2015), a variety of studies demonstrate higher prevalence of degenerative disorders, including AD, and other report neuro-protective and anti-aging effects of DHEA/DHEAS and possible corresponding mechanisms of DHEA/DHEAS actions (Hildreth *et al.* 2013, Lu *et al.* 2003, Traish *et al.* 2011, Hill *et al.* 2014a, Maggio *et al.* 2015, Starka *et al.* 2015). Furthermore, adrenal androgens serve as precursors of potent androgens or estrogens in target tissues (Labrie 2003).

Our results are in accordance with previous data indicating attenuated activity of *zona reticularis* in AD (Aldred and Mecocci 2010, Bernardi *et al.* 2000, Hillen *et al.* 2000, Kim *et al.* 2003, Nasman *et al.* 1991, Weill-Engerer *et al.* 2002). Furthermore, some authors report attenuated levels of adrenal androgens in C19 steroids and particularly their sulfates in body fluids and brain tissues of AD patients (Yanase *et al.* 1996) and others report lower DHEAS levels in subjects in which the AD developed during the prospective study. The latter authors also suggest the attenuated activity of *zona reticularis* as an important factor in the pathophysiology of AD (Ponholzer *et al.* 2009).

Concerning the extra-adrenal factors influencing the balance between sulfated and unconjugated steroids, SULT2A1 is also highly expressed in the liver and have an important detoxification function in the liver metabolism. Patients suffering from some liver disorders such as alcohol cirrhosis exhibit suppressed SULT2A1 activity (Yalcin *et al.* 2013). Decreased SULT2A1 activity was also described in human with acute sepsis (Kim *et al.* 2004). However, none of volunteers included in our study suffered from these pathologies. Regarding the effect of polymorphisms in *SULT2A1* gene on the balance between sulfated and unconjugated steroids, some studies report no such association in healthy subjects from the general population (Goodarzi *et al.* 2007, Haring *et al.* 2013).

The certain contradiction of the study is the absence of analogous significant between group differences in the C/U of C21 steroids, which might be ascribed to lower stability in the case of C21 Δ^5 steroids as the pregnenolone and its sulfate are the precursors of all remaining steroids and undergo a rapid metabolism with high inter-individual variation in individual metabolic steps, while the sulfates of C19-steroids and particularly of their 5α-reduced catabolites are more stable. The absence of significant between group differences in the C/U of 5α-reduced pregnanolone isomers and allopregnanolone of AD may be explained by an effect of inter-individual variability in further metabolism of these steroids to the corresponding sulfated pregnanediols, which are end products of progesterone catabolism.

Some steroid C/U (pregnenolone, isopregnanolone, DHEA, epiandrosterone and 5α-androstane-3α,17β-diol) are higher in men, perhaps due to influence of testicular steroidogenesis on the balance between sulfated and unconjugated forms of the steroids. The C/U investigated mostly do not correlate with age except the C/U for androsterone, which may be ascribed to relative homogeneity of the sample as concerns the age. This may be also the reason why BMI does not significantly influence the values of the steroid C/U.

In conclusion, the present study demonstrating an association between attenuated sulfation of C19 steroids in adrenal *zona reticularis* and the pathophysiology of AD is compatible with the concept of "older" *zona reticularis* in AD patients compared with controls.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

AD, Alzheimer´s disease; ANCOVA, analysis of covariance; ANOVA, analysis of variance; BAI, body adiposity index; BMI, body mass index; CBG, corticosteroid binding globulin; C/U, conjugates to unconjugated counterparts ratio; C19, C21, carbon number of steroid; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; GABA, gamma-aminobutyric acid; GC-MS, gas chromatography-mass spectrometry; HbA1c, glycated hemoglobin (A1c); HDL, high density lipoprotein; LDL, low density lipoprotein; SHBG, sex hormone binding globulin;

SULT2A1, sulfotransferase family 2A, SULT1E1, sulfotransferase family 1E, estrogen-dehydroepiandrosterone preferring, member 1; preferring, member 1; WHR, waist to hip ratio.

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