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Effects of valproate and carbamazepine monotherapy on neuroactive steroids, their precursors and metabolites in adult men with epilepsy

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ABSTRACT

Only limited data is available concerning the role of unconjugated Δ^5 C19-steroids and almost no data exists regarding the neuroactive C21 and C19 3α-hydroxy-5α/β-metabolites in men with epilepsy. To evaluate the alterations in serum neuroactive steroids and related substances in adult men with epilepsy on valproate and carbamazepine monotherapy, we have measured 26 unconjugated steroids, 18 steroid polar conjugates, gonadotropins and sex hormone binding globulin (SHBG) in 6 and 11 patients on valproate and carbamazepine monotherapy, respectively, and in 19 healthy adult men, using the GC–MS and immunoassays. Decreased testosterone, free androgen index, free testosterone, androstenediol, 5α-androstane-3α,17β-diol (androstanediol), androsterone, epiandrosterone, DHEA, 7β-hydroxy-DHEA, and DHEAS levels were associated with epilepsy *per se*. Valproate (VPA) therapy increased 5α-dihydrotestosterone, androsterone, epiandrosterone, DHEA, DHEAS, and 7β-hydroxy-DHEA levels. Decrease in pregnenolone and 17-hydroxypregnenolone were associated with epilepsy with no effect of antiepileptic drugs (AEDs). Alternatively, the increase in progesterone levels was linked to epilepsy and VPA further increased progesterone levels. Reduced steroid 20α-hydroxy-metabolites and cortisol were connected with epilepsy without an effect of AEDs. Carbamazepine induced only slight decrease in isopregnanolone, 5α,20α-tetrahydroprogesterone, and androstanediol levels.

1. Introduction

Various adrenal and gonadal steroids can cross the blood–brain barrier [1]. Besides the binding to intracellular receptors in brain, some steroids, their metabolites as well as locally produced brain steroids (which are known as neurosteroids) can bind to active sites of neuronal membrane receptors and influence the ion transport and neuronal activity [2–8]. The neurosteroids and steroid neuromodulators of peripheral origin are known as neuroactive steroids (NAS). Several NAS increase neuronal activity and consequently the cognitive abilities and memory. Some of these substances may also increase the neuronal excitability and frequency of epileptic seizures. Estradiol influences synaptic connectivity and increases the neuronal excitability [8,9] but could also act as a neuroprotective substance like dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA) and its 7-hydroxy and 7-oxo metabolites [10–15]. Pregnenolone sulfate (PregS) may be either excitotoxic or neuroprotective, depending on the type of neurotransmitter receptor-associated channels to which it binds [16,17].

Progesterone [18] and some of its reduced-metabolites [19] possess anticonvulsive, hypnotic and sedative effects. Besides these NAS, some reduced C19-steroids [10–14,20,21] also exert the aforementioned effects which, however, may not be a monotonous function of their concentration [22]. Sulfation of NAS or hydrolysis of their polar conjugates can invert the neuromodulatory effects of the original substances [23]. Progesterone deficiency in women with epilepsy may be associated with a lack of its neuroinhibiting

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metabolite allopregnanolone, which may correlate with a higher frequency of epileptic seizures. Several studies indicated a connection between the catamenial epilepsy and the disturbances in the biosynthesis of progesterone and its reduced-metabolites [22,24,25]. Progesterone and its derivatives are also suggested as anticonvulsant therapy [26–28]. The production of some NAS (like 3α-hydroxy-5α/β-androstanes) is closely associated with the activity of hypothalamic corticoliberin-containing neurons. Corticoliberin (CRH) is not only the principal regulator of the central hypothalamic–pituitary–adrenal (HPA) axis but also exerts direct actions on the peripheral tissues. CRH type-1 receptors (CRH1R) have been found primarily within the adrenal *zona reticularis* (ZR) [29]. CRH probably directly operates on human adrenocortical cells in addition to an intra-adrenal CRH receptor/ACTH system [29,30]. Smith et al. [31] have reported that CRH is as effective as ACTH at stimulating sulfated dehydroepiandrosterone (DHEAS) production in adrenals but is 70% less potent than ACTH at stimulating adrenal cortisol production. Mesiano and Jaffe [32] have shown that by the 30th week of gestation, the definitive and transition zones of the fetal adrenal begin to resemble the adult *zona glomerulosa* and *zona fasciculata*, respectively. The adrenal fetal zone primarily producing conjugated Δ^5 C19-steroids is similar to the adult *zona reticularis* but unlike the latter, the fetal zone also produces excessive amounts of conjugated C21 Δ^5 steroids [33].

In the literature only limited data is available concerning the role of unconjugated Δ^5 C19-steroids and almost no data exists regarding the neuroactive C21 and C19 3α-hydroxy-5α/β-metabolites in men with epilepsy (MWE). The only exception is the study of Brunet et al. who evaluated the effects of long-term antiepileptic therapy on the catabolism of testosterone and followed urinary excretion of androsterone, etiocholanolone and their 11β-hydroxy-metabolites [34].

The authors suggested that an induction of the hepatic synthesis of sex hormone binding globulin (SHBG) may be the mechanism by which the epileptic drugs (AEDs) decrease the levels of free testosterone in serum. The reduced excretion of androsterone and normal levels of etiocholanolone indicate that the AEDs do not produce an increase in the main catabolism pathway of testosterone.

Reddy et al. [20,35] demonstrated in mice that testosterone-derived neurosteroid 5α-androstane-3α,17β-diol (androstanediol) has powerful protective effect against seizures induced by $GABA_A$-receptor ($GABA_A$-r) antagonists. The authors suggested that androstanediol could be an endogenous modulator of seizure susceptibility in men with epilepsy [20]. Anticonvulsant properties were also reported for androsterone and etiocholanolone [21]. Although of lower potency, these steroids are present in relatively high amounts particularly in the sulfated forms reaching micromolar concentrations [36]. Whereas the sulfated 3α-hydroxy-5α/β-metabolites are inactive they might be locally hydrolyzed to active unconjugated substances, which operate as endogenous modulators of seizure susceptibility.

Treatment with AEDs commonly influences the steroid metabolome. Carbamazepine (CBZ), phenytoin and phenobarbitone induce the hepatic P450 cytochrome enzyme system and stimulate steroid clearance. In addition, concomitant treatment with benzodiazepines, probably acting via the $GABA_A$-r can alter the ACTH/cortisol response to stressful stimuli. Direct and indirect evidence suggest that benzodiazepines, acetazolamide and magnesium sulfate can also interfere with the renin–angiotensin–aldosterone system [37].

CBZ is known as a substance inducing impairment of the male reproductive system. Some of these effects, however, appear to be reversible [38]. CBZ therapy suppresses sperm concentration, reduces the motility of sperm [39], and negatively correlates with the sexual function score as reported by Herzog et al. [40]. Their more recent study, however, did not confirm this relationship [41].

Concerning the effects of valproate (VPA) on the levels of steroids and related substances, except Røste et al. [42], who found higher LH levels in VPA treated male patients than in the control group, most authors did not find an effect of VPA on the LH levels [38,43–45].

Although, some studies reported no effect of VPA on FSH levels [38,43], most studies found lower FSH levels in VPA treated patients than in controls [42,46] or even suppression of FSH by VPA therapy [44,45]. In addition, no effect of VPA on the LH/FSH ratio was reported by Stephen et al. [47].

Most authors found a positive correlation between CBZ treatment and gonadotropin levels [42–44,48,49], but there are also studies reporting no significant effect [38,50].

Valproate belongs to the group of enzyme non-inducing drugs which does not influence testosterone levels in men [38,42–47,51]. VPA therapy appears to have no effect on the sex hormone binding globulin (SHBG) levels [38,44,46,47,51]. Nevertheless, VPA therapy has negative effect on the testicular volume, decreases the motility of sperm, and also increases the frequency of sperm abnormalities [39]. On the other hand, some of the negative effects reported in the abovementioned study were also found for CBZ-treated group. It appears that besides the effects of AEDs, some of these consequences might be rather connected with epilepsy.

The goal of the present study was to compare the alterations in steroid metabolome (Figs. 1 and 2) in adult MWE induced by VPA monotherapy and CBZ monotherapy and to compare the steroid metabolome in these two groups with the metabolome in age-matched controls.

2. Experimental

2.1. Subjects

Seventeen adult men with epilepsy and 19 age-matched controls participated in the study. Six patients suffering from focal epilepsy were treated with CBZ. From the 11 patients on VPA therapy 4 and 7 suffered from focal and generalized epilepsy, respectively. None of the subjects included in our study had mesiotemporal lobe epilepsy. All patients were on stable AED dosage, most of them being seizure free for more than one year. The patients were treated with CBZ or VPA monotherapy for 22–168 months; in 4 of CBZ group and 8 of VPA group as this was their first antiepileptic drug.

The study subjects did not use any drug known to interfere with the steroid biosynthesis and catabolism and did not have any other endocrine disorder. All participants were non-smokers and did not consume more than one alcoholic beverage per week. Epilepsy onset occurred between 15th and 49th years of age and lasted from 5 to 23 years. No patient was sampled less than 3 months following the last seizure, most of them being seizure free for several years. After signing informed consent form approved by the Ethics Committee of the Institute of Endocrinology, all participants underwent blood sampling. For the evaluation of analytes 5 mL of blood was withdrawn on fasting in the morning. Blood samples were centrifuged and stored at −20 °C until analyzed.

2.2. Methods

Most of the steroids and their polar conjugates were measured using the previously described GC–MS method [52]. The 17-hydroxy-pregnenolone was measured by RIA as described in our previous report [53] and conjugated 17-hydroxy-pregnenolone was measured using the same method after hydrolysis as described elsewhere [52]. Estradiol was measured by RIA kit from Orion, Finland (intra-assay CV = 4.4%, inter-assay CV = 4.6%) and 17-

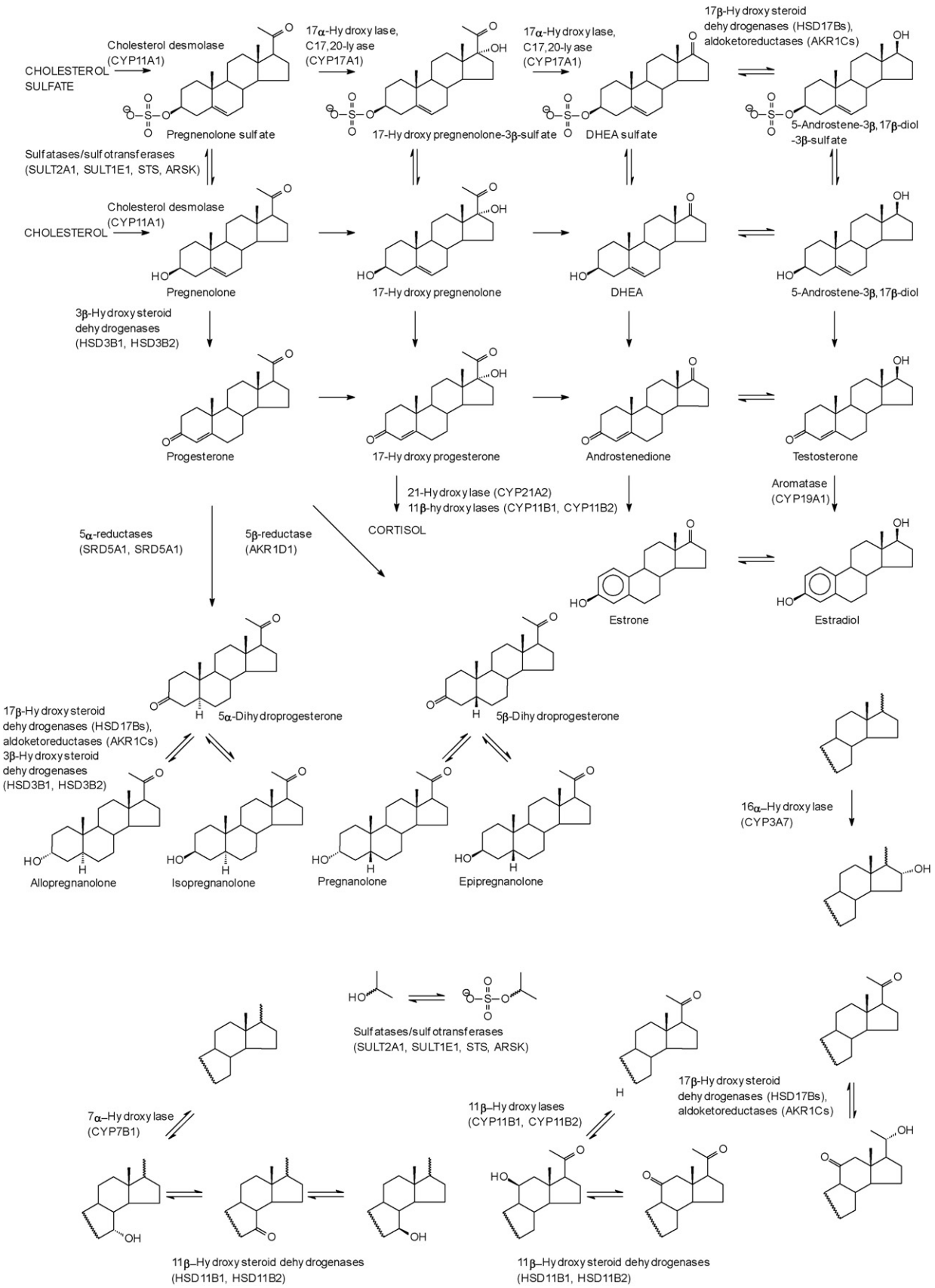

Fig. 1. Simplified scheme of steroid biosynthesis.

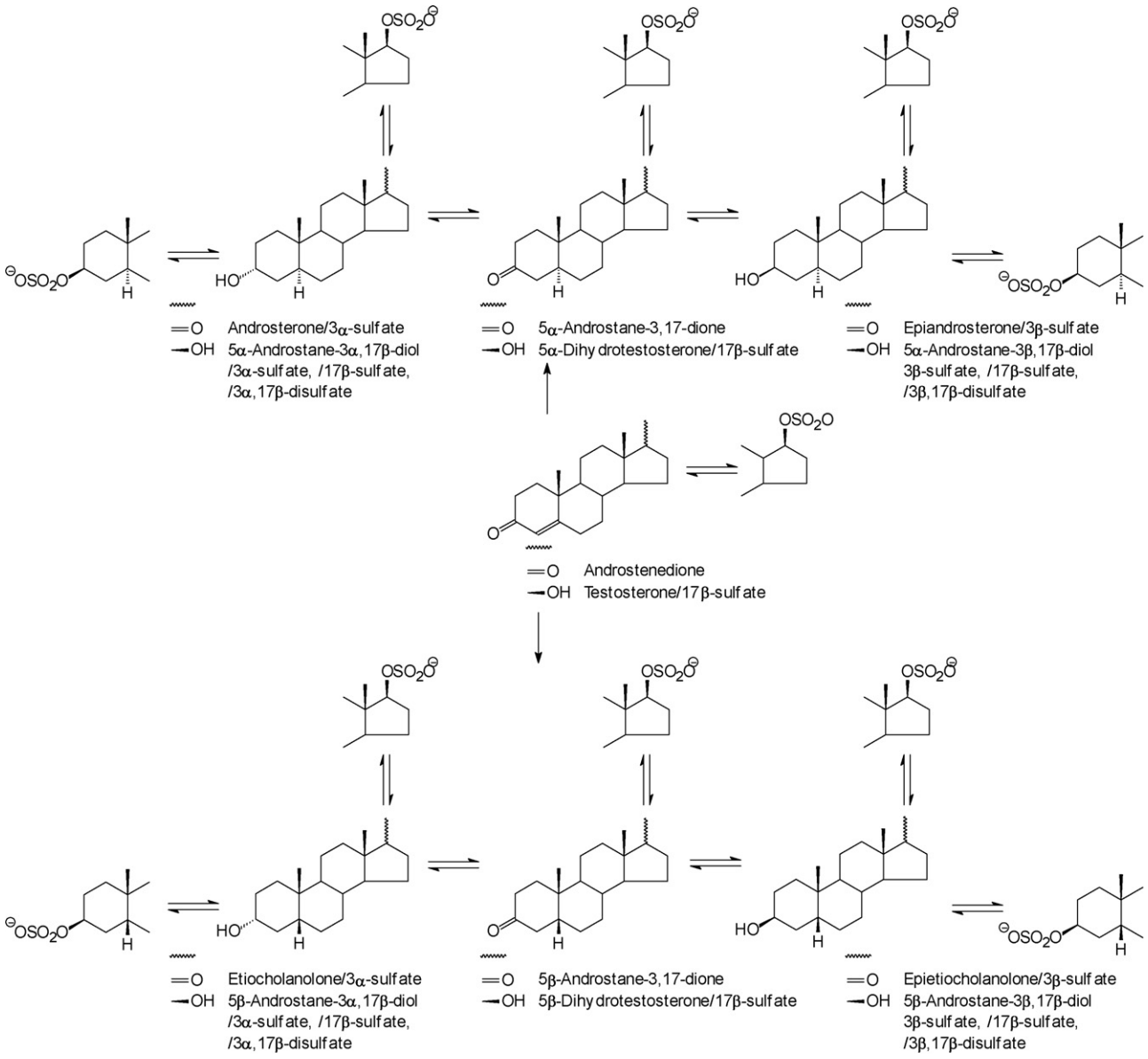

Fig. 2. Simplified scheme of the biosynthesis of reduced 5α/β-androstane metabolites.

hydroxy-progesterone was assessed by kit from Immunotech, France (intra-assay CV = 5.2%, inter-assay CV = 6.5%). Cortisol was analyzed using RIA kit from Orion, Finland (intra-assay CV = 3.8%, inter-assay CV = 4.4%), LH by IRMA kit from Immunotech, France (intra-assay CV = 3.7%, inter-assay CV = 4.3%), FSH by IRMA kit from Immunotech, France (intra-assay CV = 2.6%, inter-assay CV = 4.5%) and SHBG by IRMA kit from Orion, Finland (intra-assay CV = 6.1%, inter-assay CV = 7.9%). Free androgen index (FAI) was computed as 100 × testosterone/SHBG ratio.

2.3. Statistical data analysis

To eliminate skewed data distribution and heteroscedasticity, the original data was transformed to a Gaussian distribution by a Box–Cox transformation before further processing using the statistical software Statgraphics Centurion, Version XV from Statpoint Inc. (Herndon, VA, USA).

The differences between the controls, VPA treated patients and patients on CBZ therapy were evaluated by age-adjusted ANCOVA followed by least significant difference multiple comparisons. To separate the effect of the type and duration of epilepsy from the effects of the individual AEDs, to compare the effects of the individual AEDs and dose effects of the drugs on the anthropometric characteristics, serum steroids, gonadotropins and SHBG and to separate these effects from the hormone age relationships, we applied a multivariate regression with reduction of dimensionality, known as bidirectional orthogonal projections to latent structures (O2PLS). The O2PLS method is bidirectional and enables to predict variables constituting the matrix **Y** from variables constituting the matrix **X** and *vice versa*. The predictivity of individual variables for the model may be simply expressed as a correlation of the variable with a common predictive component. The predictive component extracts variability from the **X** and **Y**, which is shared between **X** and **Y** from variability within the matrixes **X** and **Y**, which is separated into the orthogonal components.

The transformed data underwent processing by O2PLS method [54,55], which is effective in coping with the problem of severe multicollinearity within the matrixes of both dependent and independent variables. The O2PLS enabled us to find the variables with high predictivity for the description of the relationships between

Table 1
Comparison of age and other anthropometric characteristics in control group and in the groups of carbamazepine and valproate treated adult men with epilepsy and parameters related to the type of antiepileptic drug as evaluated by one-way ANOVA (age) and age-adjusted ANCOVA (remaining parameters).

Variable	Controls	Valproate	Carbamazepine	Age-adjusted ANOVA/ANCOVA, LSD multiple comparisons
Age [years]	28 (26, 37)	26 (24, 28.5)	39 (35, 40.8)	NS, VPA–CBZ
Height [cm]	183 (180, 186)	182 (178, 184)	177 (175, 178)	G*, C-CBZ
Weight [kg]	78.3 (74, 84.3)	82 (74.5, 85.5)	82 (76.5, 98)	NS
BMI [kg/m^2]	23.3 (21.7, 24.8)	24.8 (24.2, 25.6)	26.6 (24.9, 30.6)	G*, C-CBZ
Waist [cm]	81.3 (76.2, 86.4)	92 (86.5, 98)	94 (91.3, 101)	G**, C-CBZ, C-VPA
Hip [cm]	98.4 (96.3, 102)	103 (99, 108)	107 (103, 111)	G*, C-CBZ
Waist to hip ratio	0.824 (0.79, 0.856)	0.887 (0.85, 0.921)	0.891 (0.87, 0.915)	G*, C-VPA
Dose	–	900 (550, 1225)	675 (600, 787.5)	–
Plasma level [μmol/L]	–	327 (285.4, 338.5)	29.6 (27.175, 32.1)	–
Duration of monotherapy [months]	–	75 (57.5, 110.5)	117 (79.5, 138)	NS
Age of epilepsy onset [years]	–	18 (15.5, 21)	20 (19, 30.75)	NS
Duration of epilepsy [years]	–	9 (6, 11)	11 (7.5, 17.5)	NS

NS, no factor reached significance in ANCOVA model, *$p < 0.05$, **$p < 0.01$; G, group; A, age; C, controls; VPA, patients on valproate; CBZ, patients on carbamazepine; in multiple comparison testing, only significant differences ($p < 0.05$) were shown.

X and **Y** and to find the structure of these relationships. The O2PLS model may be expressed as follows:

$$\mathbf{X} = \mathbf{T}_p\mathbf{P}_p + \mathbf{T}_0\mathbf{P}_0 + \mathbf{E}$$

$$\mathbf{Y} = \mathbf{U}_p\mathbf{Q}_p + \mathbf{U}_0\mathbf{Q}_0 + \mathbf{E}$$

where **X** is the matrix with *l* independent variables and *i* subjects, **Y** is the matrix of *m* dependent variables and *i* subjects. $\mathbf{T}_p$ and $\mathbf{T}_0$ represent the matrixes of component scores from the predictive and orthogonal components, respectively extracted from **X**. $\mathbf{P}_p$ and $\mathbf{P}_0$ represent the matrixes of component loadings from the predictive and orthogonal component, respectively extracted from **X**. Similarly, $\mathbf{U}_p$ and $\mathbf{U}_0$ represent the matrixes of component scores from the predictive and orthogonal component, respectively extracted from **Y**. $\mathbf{Q}_p$ and $\mathbf{Q}_0$ represent the matrixes of component loadings from the predictive and orthogonal component extracted from **Y**. **E** and **F** are error terms.

We have tested the relevance of individual variables for the model using a criterion Variable Importance (VIP). Only the variables that showed significant relevance for the first and/or the second predictive component were included in the model. Similarly, the relevant number of predictive components was tested using a criterion PRediction Error Sum of Squares (PRESS).

The statistical software SIMCA-P+ Version 12.0.0.0 from Umetrics AB (Umeå, Sweden) was used for data analysis. The software enabled us to find the number of the relevant components utilizing the PRediction Error Sum of Squares and also allowed the detection of multivariate non-homogeneities and testing the multivariate normal distribution and homoscedasticity.

3. Results

The primary aim of our study was to obtain a complex insight on the effects of epilepsy and antiepileptic therapy on the steroid metabolome in adult men. Due to limited data available, we have selected the approach investigating almost all important steroids and related substances instead of focusing on particular steroids. Particular attention was paid on the role of unconjugated Δ5 and neuroactive C21 and C19 3α-hydroxy-5α/β-metabolites. A number of neuroactive and neuroprotective substances in MWE were measured for the first time.

3.1. Anthropometric characteristics

Differences in anthropometric characteristics between MWE on VPA, CBZ and controls are demonstrated in Table 1. Age did not significantly differ between the control group and the groups of VPA and CBZ-treated patients but CBZ-treated patients were significantly older than those treated by VPA. Therefore, the simultaneous evaluation of differences between controls, VPA and CBZ-treated patients for all parameters except the age was performed using an age-adjusted ANCOVA. No differences between the groups were observed for body weight. CBZ patients were significantly lower than controls while VPA treated patients and controls did not differ in height. In contrast to weight, the BMI in CBZ-treated patients was higher than in controls. Both patients' groups showed significantly higher values for waist circumference. Hip circumference in CBZ group was higher than in controls while VPA group showed higher waist to hip ratio (WHR) in comparison with controls.

3.2. Gonadotropins, testosterone, 5α-dihydrotestosterone, 5α/β-androstane-3α/β-diols, and SHBG

We did not observe any change in gonadotropin levels and LH/FSH ratio in either VPA or CBZ group (Table 2). Our data showed significantly suppressed levels of total testosterone and free testosterone in both VPA and CBZ groups in comparison with the controls while in FAI the difference reached significance only for CBZ (Table 2). In contrast to testosterone, 5α-dihydrotestosterone in controls and patients on VPA did not significantly differ but was lower in CBZ-treated patients when compared to controls and VPA group (Table 2). Both VPA and CBZ groups had pronouncedly lower serum 5-androstene-3β,17β-diol (androstenediol) and androstanediol compared to the controls and did not significantly differ from each other (Table 2).

The levels of conjugated androstanediol and conjugated 5α-androstane-3β,17β-diol did not significantly differ between the groups (Table 3). However, the conjugated 5β-androstane-3β,17β-diol was significantly lower in the CBZ group, while no difference was noticed for the controls and the VPA group (Table 3).

The results of the present study did not show any difference in SHBG levels between MWE and healthy controls and no effect was found of either VPA or CBZ (Table 2).

Table 2
Serum unconjugated steroids, gonadotropins and sex hormone binding globulin in control group and groups of valproate and carbamazepine treated adult men with epilepsy as evaluated by age-adjusted ANCOVA.

Variable	Analytical method	Controls	Valproate	Carbamazepine	Age-adjusted ANCOVA + LSD multiple comparisons
Pregnenolone [nmol/L]	GC–MS	1.85 (1.16, 2.97)	0.694 (0.533, 0.834)	0.424 (0.391, 0.52)	G***, C-VPA, C-CBZ, VPA–CBZ
17-Hydroxypregnenolone [nmol/L]	RIA	10.3 (5.6, 23.8)	6.34 (3.81, 9.37)	4.88 (2.61, 7.57)	NS
Dehydroepiandrosterone [nmol/L]	GC–MS	13.2 (8.72, 18.2)	9.66 (7.6, 13.5)	6.84 (3.75, 7.26)	G**, C-CBZ
Androstenediol [nmol/L]	GC–MS	3.24 (2.61, 3.72)	1.6 (0.99, 2.14)	0.899 (0.774, 1.02)	G***, C-VPA, C-CBZ
Progesterone [nmol/L]	GC–MS	0.846 (0.564, 1.07)	1.26 (1.21, 1.84)	1.28 (1.16, 1.52)	G***, C-VPA, C-CBZ
17-Hydroxyprogesterone [nmol/L]	RIA	2.65 (2.1, 3.16)	3.65 (2.52, 5.68)	2.82 (2.7, 3.94)	NS, C-VPA
Androstenedione [nmol/L]	GC–MS	3.28 (2.34, 4.47)	4.12 (2.77, 5.39)	2.19 (1.82, 2.49)	G*, C-CBZ, VPA–CBZ
Testosterone [nmol/L]	GC–MS	15.4 (11.2, 18.2)	8.43 (6.92, 11.6)	7.45 (6.45, 8.39)	G**, C-VPA, C-CBZ
Free androgen index	–	49 (35.7, 62.3)	35.2 (25.8, 64.6)	29.4 (21.2, 34.9)	G*, C-CBZ
Free testosterone [pmol/L]	–	348 (236, 438)	194 (155, 242)	158 (127, 181)	G***, C-VPA, C-CBZ
Allopregnanolone [nmol/L]	GC–MS	0.189 (0.154, 0.205)	0.182 (0.156, 0.223)	0.0953 (0.0792, 0.112)	G**, C-CBZ, VPA–CBZ
Isopregnanolone [nmol/L]	GC–MS	0.317 (0.284, 0.433)	0.154 (0.11, 0.181)	0.0683 (0.0622, 0.0949)	G***, C-VPA, C-CBZ, VPA–CBZ
Pregnanolone [nmol/L]	GC–MS	0.0476 (0.0427, 0.0555)	0.0844 (0.0708, 0.126)	0.00914 (0.0079, 0.0353)	G***, C-VPA, C-CBZ, VPA–CBZ
20α-Dihydropregnenolone [nmol/L]	GC–MS	1.54 (1.27, 1.82)	0.869 (0.668, 0.924)	0.495 (0.342, 0.571)	G***, C-VPA, C-CBZ, VPA–CBZ
20α-Dihydroprogesterone [nmol/L]	GC–MS	1.3 (0.846, 1.71)	0.631 (0.271, 0.981)	0.259 (0.136, 0.387)	G***, C-VPA, C-CBZ
5α,20α-Tetrahydroprogesterone [nmol/L]	GC–MS	1.59 (1.24, 2.44)	0.559 (0.313, 0.608)	0.183 (0.158, 0.26)	G***, C-VPA, C-CBZ, VPA–CBZ
5α-Dihydrotestosterone [nmol/L]	GC–MS	0.665 (0.469, 0.901)	0.595 (0.51, 0.783)	0.35 (0.269, 0.521)	G*, C-CBZ, VPA–CBZ
Androsterone [nmol/L]	GC–MS	0.417 (0.369, 0.573)	0.442 (0.341, 0.53)	0.193 (0.148, 0.247)	G***, C-CBZ, VPA–CBZ
Epiandrosterone [nmol/L]	GC–MS	0.44 (0.304, 0.647)	0.399 (0.335, 0.556)	0.242 (0.161, 0.263)	G**, C-CBZ, VPA–CBZ
Etiocholanolone [nmol/L]	GC–MS	0.12 (0.0954, 0.159)	0.522 (0.162, 2.69)	0.718 (0.0553, 1.48)	G*, C-VPA
Epietiocholanolone [nmol/L]	GC–MS	0.00477 (0.00367, 0.00534)	0.0165 (0.00387, 0.0224)	0.00582 (0.0012, 0.00986)	NS, C-VPA
5α-Androstane-3α,17β-diol [nmol/L]	GC–MS	0.443 (0.372, 0.517)	0.14 (0.137, 0.2)	0.108 (0.0973, 0.164)	G***, C-VPA, C-CBZ
7α-Hydroxy-DHEA [nmol/L]	GC–MS	1.64 (1.31, 1.93)	1.71 (1.4, 2.33)	0.991 (0.792, 1.19)	G*, C-CBZ, VPA–CBZ
7β-Hydroxy-DHEA [nmol/L]	GC–MS	0.426 (0.32, 0.529)	0.47 (0.317, 0.61)	0.233 (0.183, 0.251)	G*, C-CBZ, VPA–CBZ
5-Androstene-3β,7α,17β-triol [nmol/L]	GC–MS	0.364 (0.291, 0.431)	0.137 (0.0878, 0.186)	0.0956 (0.0614, 0.128)	G***, C-VPA, C-CBZ
5-Androstene-3β,7β,17β-triol [nmol/L]	GC–MS	0.433 (0.351, 0.522)	0.088 (0.0596, 0.115)	0.0476 (0.0332, 0.0585)	G***, A**, C-VPA, C-CBZ
Cortisol [nmol/L]	RIA	309 (262, 370)	187 (171, 219)	191 (168, 239)	G***, C-VPA, C-CBZ
Lutropin	IRMA	3.8 (2.9, 5.2)	4.03 (2.3, 5.53)	3.06 (2.06, 4.33)	NS
Follitropin	IRMA	3.9 (2.75, 4.8)	2.52 (1.79, 3.72)	4.32 (3.01, 5.56)	A*
Lutropin/follitropin	–	1.08 (0.853, 1.73)	1.34 (0.79, 1.93)	0.61 (0.482, 0.792)	NS
Sex hormone binding globulin	IRMA	30.3 (21.1, 40.5)	22.7 (15.3, 40)	31.1 (16.8, 36.5)	NS

G, group; A, age; C, controls; VPA, patients on valproate; CBZ, patients on carbamazepine, C-VPA, C-CBZ and VPA–CBZ symbolize significant differences between individual groups as evaluated by LSD multiple comparisons ($p < 0.05$).

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Table 3
Serum steroid polar conjugates in control group and groups of valproate and carbamazepine treated adult men with epilepsy as evaluated by age-adjusted ANCOVA.

Variable	Analytical method	Controls	Valproate	Carbamazepine	Age-adjusted ANCOVA + LSD multiple comparisons
Conjugated pregnenolone [nmol/L]	GC–MS	127 (103, 154)	112 (82.4, 130)	79.2 (66.4, 90.1)	G**, A*, C-CBZ
Conjugated 17-hydroxypregnenolone [nmol/L]	GC–MS	21.8 (14.1, 32.2)	36.7 (23.8, 50.7)	11.5 (8.19, 14.4)	G**, A**, C-VPA, VPA–CBZ
Conjugated dehydroepiandrosterone [nmol/L]	GC–MS	2940 (2330, 3380)	2620 (1310, 3460)	1590 (855, 1850)	G*, C-CBZ
Conjugated androstenediol [nmol/L]	GC–MS	303 (243, 494)	743 (377, 992)	380 (316, 481)	G*, C-VPA
Conjugated allopregnanolone [nmol/L]	GC–MS	4.53 (3.57, 5.97)	6.13 (3.8, 11.5)	3.95 (2.76, 5.47)	NS, C-VPA
Conjugated isopregnanolone [nmol/L]	GC–MS	9.08 (7.83, 10.5)	12.4 (8.54, 18)	7.86 (7.38, 8.22)	NS
Conjugated pregnanolone [nmol/L]	GC–MS	9.26 (8.36, 17.7)	20.4 (17.9, 27.2)	14.7 (10.2, 18.1)	G*, C-VPA
Conjugated epipregnanolone [nmol/L]	GC–MS	0.536 (0.38, 0.62)	2.36 (2, 3.32)	1.48 (1.1, 1.78)	G***, C-VPA, C-CBZ, VPA–CBZ
Conjugated 20α-dihydropregnenolone [nmol/L]	GC–MS	544 (485, 644)	350 (246, 427)	224 (203, 296)	G***, C-VPA, C-CBZ, VPA–CBZ
Conjugated 5β-pregnane-3α,20α-diol [nmol/L]	GC–MS	25.8 (20.5, 36.5)	21.5 (10.7, 40.6)	16 (8.04, 29.3)	NS, C-VPA
Conjugated 5β-pregnane-3β,20α-diol [nmol/L]	GC–MS	13.9 (8.56, 20.4)	8.63 (6.41, 14)	4.36 (3.83, 8.69)	NS, C-CBZ
Conjugated androsterone [nmol/L]	GC–MS	920 (617, 1080)	801 (496, 2110)	274 (127, 403)	C-CBZ, VPA–CBZ
Conjugated epiandrosterone [nmol/L]	GC–MS	342 (211, 532)	336 (237, 751)	159 (65.3, 250)	NS
Conjugated etiocholanolone [nmol/L]	GC–MS	60.1 (32.2, 69.9)	77.8 (45.8, 116)	27.1 (16.2, 57.9)	NS, VPA–CBZ
Conjugated epietiocholanolone [nmol/L]	GC–MS	17.9 (11.8, 42.4)	37.5 (22.3, 53)	7.24 (4.26, 12.3)	NS, VPA–CBZ
Conjugated 5α-androstane-3α,17β-diol [nmol/L]	GC–MS	74.2 (35.3, 121)	58.7 (33.6, 94.6)	34.9 (33.4, 44)	NS
Conjugated 5α-androstane-3β,17β-diol [nmol/L]	GC–MS	67.7 (33.7, 103)	49 (40.8, 113)	27.2 (16.5, 46.2)	NS
Conjugated 5β-androstane-3β,17β-diol [nmol/L]	GC–MS	0.882 (0.727, 1.18)	0.908 (0.691, 1.18)	0.563 (0.344, 0.668)	NS, C-CBZ, VPA–CBZ

G, group; A, age; C, controls; VPA, patients on valproate; CBZ, patients on carbamazepine, C-VPA, C-CBZ and VPA–CBZ symbolize significant differences between individual groups as evaluated by LSD multiple comparisons ($p < 0.05$).

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

3.3. Other C19-steroids

Androstenedione levels were suppressed in the CBZ group in comparison with both VPA group (showing the highest values) and controls. Controls did not significantly differ from the VPA group (Table 2).

DHEA and DHEAS levels were significantly lower in the CBZ group in comparison with the control group, while the VPA group did not significantly differ from the latter (Table 2). The levels of the 7α/β-hydroxy-metabolites of DHEA reflected the levels of the parent steroid. The metabolites, however, were significantly reduced in the CBZ group not only when compared with the control group but also in comparison with the VPA group being very close to the control group (Table 2).

Like the DHEA, the unconjugated androstenediol exhibited decreasing trend from controls to the CBZ group (controls > VPA > CBZ) (Table 2). On the contrary, conjugated androstenediol was highest in the VPA group, which significantly differed from the control group. Controls (showing the lowest values) and CBZ group did not significantly differ from each other (Table 3).

Androsterone and epiandrosterone levels were lower in the CBZ group than in VPA or control groups (Table 2).

3.4. Δ^5 C21 steroids

We observed a decreasing trend from controls to the CBZ group (controls > VPA > CBZ) for pregnenolone in which all groups significantly differed from each other and for 17-hydroxypregnenolone, in which, however, the differences did not reach a significance (Table 2). Conjugated pregnenolone was lower in the CBZ-treated MWE but showed no difference between controls and the VPA group (Table 3). Conjugated 17-hydroxypregnenolone was highest in the VPA group, which significantly differed from the controls and from the CBZ group (showing lowest levels for the latter group) (Table 3).

3.5. Δ^4 C21 steroids

Almost the same progesterone levels were found for both CBZ and VPA group and both groups had significantly higher progesterone levels than controls. 17-Hydroxyprogesterone levels were highest in the VPA group, which significantly differed from the controls (Table 2). Despite very close median concentrations of the steroid in the control and CBZ groups the difference between VPA and CBZ groups did not reach significance. MWE had suppressed cortisol levels in both VPA and CBZ groups (Table 2).

3.6. Steroid 20α-hydroxy-metabolites

All steroid 20α-hydroxy-metabolites showed a decreasing trend from controls to the CBZ group (controls > VPA > CBZ). For conjugated and unconjugated 20α-dihydropregnenolone as well as for 5α,20α-tetrahydroprogesterone all groups significantly differed from each other, while for 20α-dihydroprogesterone, the difference between the VPA group and CBZ group did not reach a significance (Tables 2 and 3).

3.7. C21 5α/β-reduced-metabolites

The unconjugated 5α-pregnanolone isomers were pronouncedly suppressed in the CBZ group when compared to controls and to the VPA group. While allopregnanolone showed no difference between the VPA group and controls, the 3β-metabolite isopregnanolone was significantly lower in VPA group than in the control group. The unconjugated 5β-isomer pregnanolone showed highest values in VPA group, which significantly differed from the controls and CBZ group. The CBZ-treated patients had significantly lower pregnanolone levels than controls (Table 2).

The conjugated pregnanolone isomers were highest in the VPA group showing a decreasing trend from the VPA group to the CBZ group (VPA > control > CBZ) and, except for the conjugated isopregnanolone, significantly differed from the controls. In respect of conjugated epipregnanolone all groups significantly differed from each other (Table 3).

3.8. C19 5α/β-reduced-17-oxo-metabolites

While the 5α-reduced-17-oxo-metabolites did not differ between the controls and VPA group, their levels in the CBZ-treated patients were significantly lower than in the remaining groups. The only exception was conjugated epiandrosterone for which the differences did not reach significance. Alternatively, the unconjugated 5β-isomers showed significantly higher levels in the VPA group when compared with the controls. The conjugated 5β-isomers were significantly higher in VPA group when compared to the CBZ group (Tables 2 and 3).

3.9. Differentiation between contributions of epilepsy indices and antiepileptic drugs to alterations in serum steroids, outcomes of multivariate regression

To separate the effects of epilepsy indices from the effects of AEDs on the steroid metabolome, we have used multivariate regression with reduction of dimensionality (for details see Section 2.3). The variability contained in the presence or absence of epilepsy indices, use or not use of particular AEDs, and age of the participants (the first group of variables constituting matrix **X**) and serum steroids, anthropometric indices (except of age), gonadotropins and SHBG (the second group of variables constituting matrix **Y**) was fragmentized into two mutually independent predictive components each explaining a part of variability, which is shared between the **X** and **Y**. Table 4 and Fig. 3 show the significance of each variable for explanation of the relationships between **X** and **Y**. The first and the second predictive component explained 38.4% and 20.0% of the total variability, respectively. The first predictive component explained the effect of epilepsy indices on serum steroids, anthropometric indices (except of age), gonadotropins and SHBG, while the second predictive component explained the effect of AEDs on the same parameters.

The component loadings for the first predictive component show that the epilepsy is the most representative parameter in the **X**. The loadings for the first predictive component for the **Y** show that epilepsy is linked to significantly higher waist to hip ratio, significantly lower height and significantly lower levels of pregnenolone, 17-hydroxypregnenolone, DHEA, androstenediol, testosterone, FAI, free testosterone, isopregnanolone, 20α-dihydropregnenolone, 20α-dihydroprogesterone, 5α,20α-tetrahydroprogesterone, 5α-dihydrotestosterone, androsterone, epiandrosterone, androstanediol, 7β-hydroxy-DHEA, 5-androstene-3β,7α,17β-triol, 5-androstene-3β,7β,17β-triol, cortisol, conjugated pregnenolone, DHEAS, and conjugated 20α-dihydropregnenolone, and significantly higher levels of progesterone and conjugated epipregnanolone.

As apparent from the component loadings, the second predictive component positively correlates with indices linked to VPA therapy and negatively with those connected with CBZ use. Although the epilepsy presence also positively correlates with the second predictive component, the corresponding component loading is much less than those for the variables related to individual AEDs. Thus the second predictive component principally discriminates between CBZ and VPA treatment. For instance, the positive correla-

Table 4
Relationships between epilepsy status, duration of epilepsy, type of epilepsy, type of treatment, dose effects of antiepileptic drugs (valproate/carbamazepine), age of the participants and serum levels of steroids, gonadotropins and sex hormone binding globulin as calculated using the O2PLS model (for details see Section 2.3).

Variable	Component 1	Component of epilepsy anthropometric parameters and steroids			Component 2	Component of epilepsy and steroids			
	Component loading	Component loading/95%CI	Component loading/99%CI	R^a	Component loading	Component loading/95%CI	Component loading/99%CI	R^a	
*Epilepsy indices (matrix **X**)*									
Epilepsy	0.483	5.32	3.36	0.842	**	0.172	1.55	0.98	0.436	*
Duration of epilepsy	0.465	2.95	1.87	0.720	**	0.012	0.03	0.02	0.288	NS
Focal epilepsy	0.416	4.63	2.92	0.687	**	−0.188	−0.70	−0.44	−0.034	NS
Generalized epilepsy	0.139	2.29	1.45	0.285	**	0.430	2.02	1.28	0.589	**
VPA therapy	0.232	2.82	1.78	0.404	**	0.503	2.51	1.58	0.725	**
VPA, dose	0.228	3.15	1.99	0.314	**	0.444	1.63	1.03	0.716	**
CBZ therapy	0.360	4.08	2.58	0.630	**	−0.391	−1.22	−0.77	−0.312	*
CBZ, dose	0.357	4.19	2.65	0.625	**	−0.388	−1.40	−0.89	−0.332	*
*Relevant steroids and anthropometric characteristics (matrix **Y**)*									
Height	−0.115	−1.62	−1.02	−0.414	**	0.118	0.46	0.29	0.189	NS
Waist to hip ratio	0.138	1.42	0.90	0.495	*	0.020	0.05	0.03	0.032	NS
Pregnenolone	−0.239	−7.95	−5.02	−0.859	**	−0.024	−0.21	−0.13	−0.039	NS
17-OH-pregnenolone	−0.145	−1.81	−1.14	−0.519	**	0.161	0.70	0.44	0.259	NS
DHEA	−0.219	−2.11	−1.33	−0.785	**	0.228	2.90	1.83	0.368	**
Androstenediol	−0.236	−7.05	−4.45	−0.847	**	0.013	0.13	0.08	0.020	NS
Progesterone	0.141	1.32	0.83	0.507	*	0.243	1.09	0.69	0.392	*
17-OH-progesterone	0.071	0.77	0.48	0.256	NS	0.163	0.49	0.31	0.263	NS
Testosterone	−0.212	−2.51	−1.59	−0.760	**	0.070	0.27	0.17	0.112	NS
Free androgen index	−0.154	−1.67	−1.05	−0.553	**	0.066	0.28	0.18	0.106	NS
Free testosterone	−0.226	−6.25	−3.95	−0.810	**	0.083	0.34	0.22	0.133	NS
Isopregnanolone	−0.251	−7.25	−4.58	−0.901	**	−0.086	−0.58	−0.37	−0.139	NS
20α-Dihydropregnenolone	−0.219	−5.62	−3.55	−0.785	**	−0.029	−0.13	−0.08	−0.047	NS
20α-Dihydroprogesterone	−0.227	−4.23	−2.67	−0.817	**	0.076	0.54	0.34	0.122	NS
5α,20α-Tetrahydroprogesterone	−0.230	−2.95	−1.86	−0.827	**	−0.101	−0.65	−0.41	−0.164	NS
5α-Dihydrotestosterone	−0.148	−1.72	−1.09	−0.532	**	0.270	1.82	1.15	0.435	**
Androsterone	−0.197	−2.12	−1.34	−0.706	**	0.263	6.57	4.15	0.423	**
Epiandrosterone	−0.196	−1.65	−1.04	−0.703	**	0.314	4.70	2.97	0.506	**
5α-Androstane-3α,17β-diol	−0.210	−7.31	−4.62	−0.753	**	−0.102	−0.55	−0.35	−0.165	NS
7β-Hydroxy-DHEA	−0.195	−2.37	−1.50	−0.699	**	0.355	3.50	2.21	0.572	**
5-Androstene-3β,7α,17β-triol	−0.260	−6.40	−4.05	−0.932	**	−0.021	−0.25	−0.16	−0.035	NS
5-Androstene-3β,7β,17β-triol	−0.264	−7.75	−4.90	−0.950	**	−0.121	−1.39	−0.88	−0.196	*
Cortisol	−0.156	−3.42	−2.16	−0.553	**	0.014	0.06	0.04	0.065	NS
Conjugated pregnenolone	−0.147	−2.74	−1.73	−0.528	**	0.174	0.71	0.45	0.280	NS
Conjugated DHEA	−0.142	−2.51	−1.59	−0.510	**	0.140	0.43	0.27	0.224	NS
Conjugated androstenediol	0.058	0.85	0.54	0.208	NS	0.311	0.89	0.56	0.501	NS
Conjugated pregnanolone	0.051	0.89	0.56	0.184	NS	0.437	1.96	1.24	0.706	**
Conjugated epipregnanolone	0.148	3.35	2.11	0.532	**	0.424	4.60	2.91	0.683	**
Conjugated 20α-dihydropregnenolone	−0.211	−3.00	−1.90	−0.756	**	0.045	0.31	0.20	0.073	NS
Explained variability	38.4% (32.2% after cross-validation)					20.0% (18.5% after cross-validation)				

[a] *R*, component loading expressed as a correlation coefficient with the predictive component; NS, not significant.
[*] $p < 0.05$.
[**] $p < 0.01$.

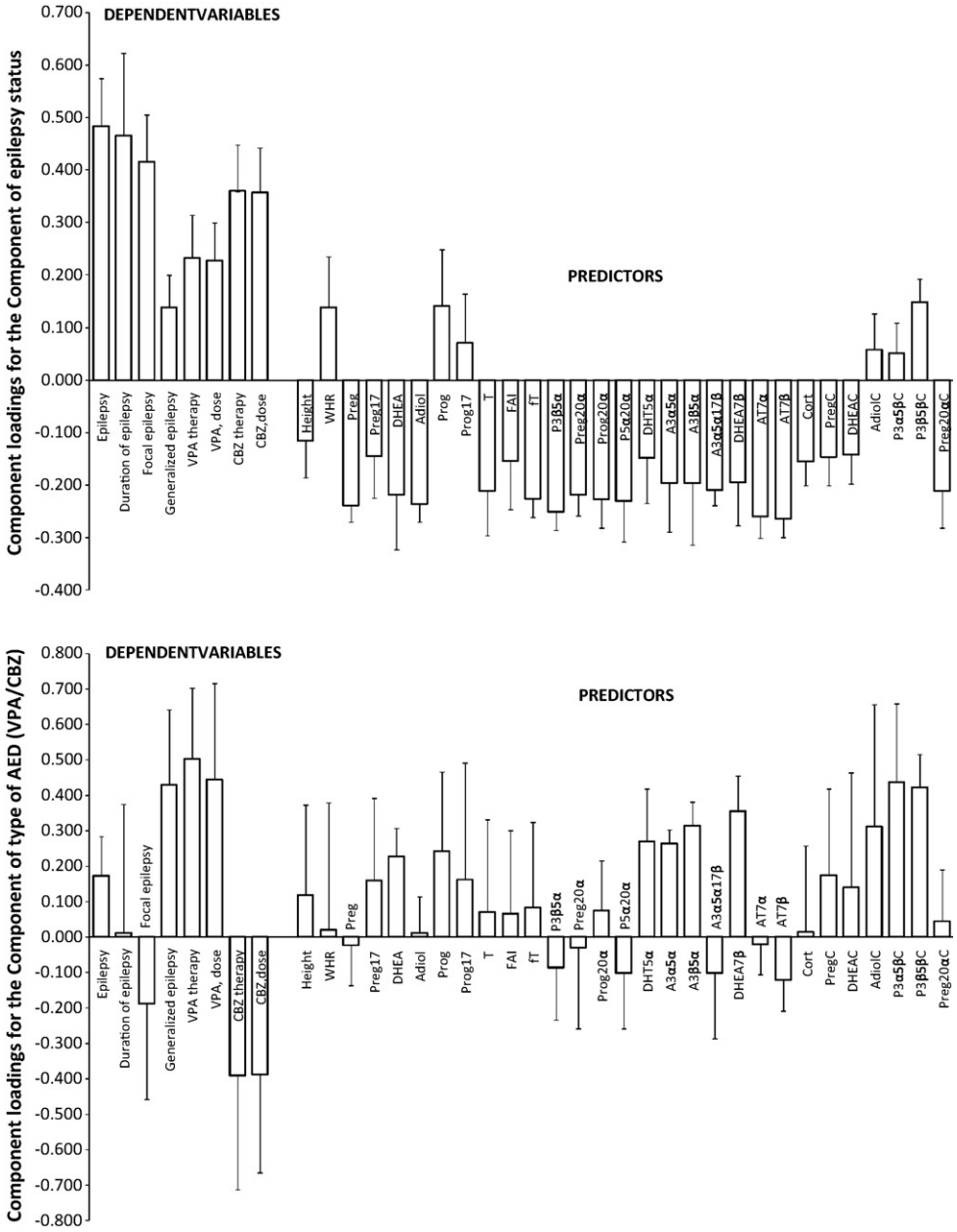

Fig. 3. Simplified scheme of steroid biosynthesis. The differentiation between the effect of the type and duration of epilepsy and the effects of the individual AEDs with the use of multivariate regression with reduction of dimensionality (the method of bidirectional orthogonal projections to latent structures, O2PLS). The bars with error bars represent component loadings with predictive components (in the form of regression coefficients) for individual variables. These components separate the variability that is shared between predictors and dependent variables from the variability within the matrixes of dependent and independent variables and from the unexplained variability.

tion of androsterone with the second predictive component points to higher levels of androsterone in VPA treated patients when compared with those using CBZ. This is consistent with the results of ANOVA testing followed by multiple comparisons (Table 2). The group factor is significant ($p < 0.001$) and the difference between VPA and CBZ groups is significant as well ($p < 0.05$). The median value of androsterone in VPA group is actually more than twice as high as that for the CBZ group.

The component loadings for the second predictive component show that valproate therapy *per se* is more important for the prediction of serum steroids than the dose of VPA. This means that dose effect is not manifested for VPA. The situation for the CBZ is similar but the confidence interval in the CBZ dose is narrower than in the CBZ therapy. This means that there might be a limited dose effect in the CBZ-treated patients. The component loadings for the second predictive component in the variables constituting **Y** show that the VPA therapy is associated with significantly higher levels of DHEA, progesterone, 5α-dihydrotestosterone, androsterone, epiandrosterone, 7β-hydroxy-DHEA, conjugated pregnanolone and conjugated epipregnanolone and significantly lower levels of 5-androstene-3β,7β,17β-triol.

4. Discussion

A study investigating almost all important steroids including androgens, progesterone, neuroactive and neuroprotective steroids, steroid polar conjugates is still lacking. Therefore, we selected VPA and CBZ-treated men with epilepsy and age-matched controls, and followed a wide spectrum of analytes covering almost all steroid metabolome including 26 unconjugated steroids, 18

steroid polar conjugates and further related compounds such as gonadotropins and SHBG. We tried also to find at which level the AEDs influence the steroid metabolome, to identify the steps in the steroid metabolic pathways in periphery, which are influenced by AEDs and to estimate the consequences of these alterations. Multivariate regression model was used to separate the effects of AEDs from the effect of epilepsy.

Some of the previous studies use greater number of patients but they focused on particular steroids only (mostly on the androgens). Other authors generally used immunoanalytic methods, which are inferior in comparison with GC–MS that was mostly used in the present study. Nevertheless, the main limitation of this study is relatively low number of subjects and consequently a low power of the statistical testing. This means that there is higher probability of falsely negative results, which may explain some discrepancies between the results from multivariate regression and ANOVA. The next limitation is the absence of the group of untreated men with epilepsy and the group of patients treated by "pharmacokinetic-neutral" AEDs such as levetiracetam, gabapentin, etc. Having these data the study would have been stronger.

4.1. Gonadotropins

Our data for VPA were in agreement with some results found in the literature, which showed no effect of VPA treatment on gonadotropin levels in MWE [38,43]. Like in the study of Stephen et al. [47] we found no effect of VPA on the LH/FSH ratio. On the other hand, Røste et al. [42] reported higher LH levels in VPA treated patients than in the control group and some authors reported lower FSH levels in VPA treated patients than in controls [42,44–46].

Concerning the effect of CBZ on LH levels, our results agreed with the data presented by Bauer et al. [50] and Lossius et al. [38] who found no significant effect of CBZ on the serum LH but were in contrast to the data of other authors reporting a positive correlation between CBZ treatment and gonadotropin levels [42–44,48,49].

4.2. Testosterone, 5α-dihydrotestosterone, 5α/β-androstane-3α/β-diols, and SHBG

Our data showed significantly suppressed levels of total testosterone and free testosterone in both VPA and CBZ groups when compared with the controls while in FAI the difference reached significance only for CBZ. Both VPA and CBZ groups had pronouncedly lower levels of testosterone precursor androstenediol compared to the controls but did not differ from each other (Table 2).

As described by Isojarvi et al. [39], VPA therapy appears to have negative effect on the testicular volume, decreases the motility of sperm, and also increases the frequency of sperm abnormalities [39]. On the other hand, some of the abovementioned negative effects were also found for the CBZ-treated group. It appears that besides the effects of AEDs, some of these consequences might be rather connected with epilepsy than with the effect of AEDs as also indicated by our results. Changes in SHBG, total and free testosterone, dihydrotestosterone and androstenedione appear to be independent of the epileptic syndrome type [56]. VPA belongs to the group of enzyme non-inducing drugs and the data in the literature shows that VPA therapy does not significantly influence testosterone levels in men [38,42–47,51] and has no effect on SHBG levels [38,44,46,47,51].

In contrast to VPA treatment most authors described that testosterone, free testosterone levels, free androgen index (FAI), or the levels of bioavailable testosterone were mostly reduced in the CBZ-treated MWE in comparison with controls [38,43,44,48–50,57]. Stöffel-Wagner et al. [48] also found elevated LH/testosterone ratio in CBZ-treated patients. However, there are studies either indicating no significant effect of CBZ on testosterone levels [51] or reporting reduced testosterone levels in untreated patients [40] and no significant effect of CBZ treatment was reported by Røste et al. [42]. These findings raise a presumption that there might be concomitant negative effects of CBZ therapy and epilepsy on the testicular function. This presumption is further supported by the results of Bauer et al. [50] who reported a return of serum androgens to normal after temporal lobe epilepsy surgery in men.

So far, there is no study evaluating the levels of 5α-dihydrotestosterone in epilepsy. The steroid is the most potent androgen and major neuroactive substance in adult men. Our present data showed that in contrast to testosterone, 5α-dihydrotestosterone in controls and patients on VPA did not significantly differ but was lower in CBZ-treated patients when compared to controls and VPA group. The different pattern of the effects of AEDs and epilepsy for testosterone and 5α-dihydrotestosterone might be a consequence of partly different sources of these hormones. While testosterone in adult men is primarily synthesized in testes, the adrenal contribution for the synthesis of 5α-dihydrotestosterone appears to be more important as was documented by pronouncedly decreasing 5α-dihydrotestosterone/testosterone ratio during male childhood, puberty and adolescence and constant value of this ratio in adulthood [58]. Labrie et al. [59] reported that 25–50% of 5α-dihydrotestosterone is still present in the prostate after castration.

In males, testosterone and 5α-dihydrotestosterone appear to enhance the development of amygdala-kindled seizures, which may have potential therapeutic value for males with epilepsy [9]. On the other hand, 5α-dihydrotestosterone blocks NMDA-type glutamate transmission and may be responsible for anti-seizure effects [60]. Therefore, the suppression of this neuroactive steroid by CBZ as indicated by our data might exert deleterious effect in MWE. In men, testosterone effects may depend on the relative concentrations of two major testosterone metabolites that exert opposing influences on neuronal excitability: estrogen potentiates whereas 5α-dihydrotestosterone inhibits NMDA-mediated conductance [61]. Hence, Herzog [61] suggested that a combined therapy using an aromatase inhibitor along with testosterone improves sexual function and may reduce seizures in men with epilepsy.

Concerning the GABA-ergic androstanediol in MWE, the levels of bioavailable androstanediol were evaluated by RIA in a single study by Herzog et al. [41]. The authors found increased levels of the abovementioned parameter in CBZ-treated patients in comparison with controls. Our GC–MS data are different. Like in the case of androstenediol and testosterone, we found decreased levels of the androstanediol in VPA and CBZ groups in comparison with controls but no difference between the VPA and CBZ groups. Testosterone-derived neuroactive steroid androstanediol has powerful protective effect against seizures induced by $GABA_A$-receptor antagonists [20,35] and could be an endogenous modulator of seizure susceptibility in men with epilepsy [20]. Whether the reduced levels of this neurosteroid in MWE (reflecting the situation in the total testosterone levels) could play a role in the pathogenesis of epilepsy remains to be clarified. This phenomenon might contribute to the pathology of epilepsy in men.

The results of the present study did not show any difference in SHBG levels between MWE and healthy controls and no effect was reported of either VPA or CBZ. Similarly, the data in the literature indicates that VPA therapy appears to have no effect on SHBG levels [38,42,44,46,47,51]. Concerning CBZ therapy, in a number of studies CBZ enhanced serum SHBG [34,44,46,48,49,51,57,62] but the effect on serum SHBG was reversible [51]. On the other hand, there are several studies in which the effect of CBZ on SHBG did not reach significance [42,43]. No effect of the temporal lobe surgery in MWE was observed on the SHBG levels [50], which indicates that

there is probably no connection between the epilepsy and serum SHBG.

4.3. C21 steroids

To our knowledge, we measured the levels of Δ^5 C21 steroids, steroid 20α-hydroxy-metabolites and their polar conjugates in MWE for the first time. These substances generally showed a decreasing trend from controls to the CBZ group (controls > VPA > CBZ).

On the other hand, our results showed almost the same progesterone levels for both CBZ and VPA group but both groups had significantly higher progesterone levels than controls. Therefore, the data suggests that higher serum progesterone levels are associated with epilepsy but not with CBZ or VPA therapy. Concerning the progesterone levels in MWE, Rattya and colleagues published contradictory results. In their first study the authors found lower levels of progesterone in the VPA treated MWE than in the control group [46], while in the second study the authors reported an increase of progesterone levels in MWE after 1-month and 3-month treatment with VPA [44].

In the present study, 17-hydroxyprogesterone levels were highest in the VPA group, which significantly differed from the controls. There is only one available report presenting the levels of 17-hydroxyprogesterone in CBZ-treated MWE who had, in contrast to our results, lower levels of the steroid than the age-matched controls [46].

4.4. C21 5α/β-reduced-metabolites

In the literature, no information evaluating the levels of C21 5α/β-reduced-metabolites in MWE is available yet. In the present study, we measured the levels of three unconjugated pregnanolone isomers and polar conjugates of all pregnanolone isomers. The unconjugated 5α-pregnanolone isomers were pronouncedly suppressed in the CBZ group when compared to controls and to the VPA group. While the neuroprotective GABA-ergic 3α-isomer allopregnanolone showed no difference between the VPA group and controls, the inactive 3β-metabolite isopregnanolone (competing with the allopregnanolone on $GABA_A$-receptors) was significantly lower in VPA group than in the control group. The GABA-ergic unconjugated 5β-isomer pregnanolone showed highest values in VPA group, which significantly differed from the controls and CBZ group. The CBZ-treated patients had significantly lower pregnanolone levels than controls. Whereas the levels of pregnanolone isomers in men are generally low, their role in the pathogenesis of epilepsy in male patients is open to discussion.

4.5. C19 5α/β-reduced-17-oxo-metabolites

The unconjugated 5β-isomers showed significantly higher levels in the VPA group when compared with the controls. The conjugated 5β-isomers were significantly higher in VPA group when compared to the CBZ group. These results as well as the results for the analogous 5β-pregnanolone isomers indicate that VPA treatment may accelerate the catabolism of both C21- and C19-steroids via stimulation of the liver 5β-reductase (AKR1D1).

Regarding the neuroactive androstane metabolites, a direct adrenal cortical secretion accounts for a minor proportion of the serum levels of androsterone, androstanediol, and 5α-dihydrotestosterone but serum androsterone is derived mainly from adrenal cortex precursors in both sexes [36]. Brunet et al. evaluated the effects of long-term antiepileptic therapy on the catabolism of testosterone and followed urinary excretion of androsterone, etiocholanolone and their 11β-hydroxy-metabolites [34]. However, although anticonvulsant properties were demonstrated for unconjugated androsterone and etiocholanolone [21], there are no studies available evaluating serum free or conjugated 3α/β-hydroxy-5α/β-androstane-17-ones in MWE.

4.6. Cortisol

We found that MWE had suppressed cortisol levels in both VPA and CBZ groups. Our data are not in agreement with others [48,50], who did not found an effect of epilepsy and/or AEDs on serum cortisol levels. The results of the present study pointed to suppressed cortisol production in the *zona fasciculata*. Although we did not measure ACTH or CRH levels, we could hypothesize that the decreased cortisol levels could be a consequence of deficient ACTH production, which is consistent with the findings of Motta et al. [63] who described lower ACTH levels in epileptics than in controls.

4.7. Differentiation between contributions of epilepsy indices and antiepileptic drugs to alterations in serum steroids, outcomes of multivariate regression

The contribution of epilepsy indices on one hand and the effects of AEDs on steroid metabolome on the other hand are still mysterious. Therefore, we tried to decipher this enigma using the multivariate regression with reduction of dimensionality (for details see Section 2.3). This approach in all probability enabled us to separate the effects of epilepsy indices from the effects of AEDs on the steroid metabolome.

The results of multivariate regression (Table 4), which are linked to the first predictive component, illustrate the negative effect of epilepsy on some anthropometric indices, and a negative correlation of epilepsy with the levels of active androgens, neuroactive anti-seizure substances (5α-dihydrotestosterone [61], androsterone [21]), neuroprotective or potentially neuroprotective steroids (DHEA [64], 7β-hydroxy-DHEA [14], 5-androstene-3β,7α/β,17β-triols) and other neuroactive steroids (DHEAS, pregnenolone sulfate, cortisol), which might, however, increase the seizure frequency.

The effects of AEDs, which are connected with the second predictive component, indicate that the VPA therapy probably induces the formation of the following steroids: the neuroprotective DHEA [64] and its neuroprotective 7β-hydroxy-metabolite [14], anti-seizure substance 5α-dihydrotestosterone, which inhibits NMDA-mediated conductance [61], anti-seizure (GABA-ergic) androsterone [21], conjugated pregnanolone and epipregnanolone, which negatively modulate NMDA receptors [65,66], the potential precursor of anti-seizure GABA-ergic substance, conjugated pregnanolone [67]. On the other hand, CBZ therapy probably has an opposite, harmful effects in MWE.

4.8. Conclusions

In conclusion, decreased testosterone, FAI, free testosterone, androstenediol, androstanediol, androsterone and epiandrosterone, DHEA, 7β-hydroxy-DHEA, DHEAS levels appears to be associated with epilepsy *per se*. VPA therapy is related to increased 5α-dihydrotestosterone, androsterone, epiandrosterone, DHEA, 7β-hydroxy-DHEA levels. Pregnenolone and 17-hydroxypregnenolone are probably decreased due to epilepsy with no effect of therapy. On the contrary, progesterone levels are most probably increased as an effect of epilepsy and VPA therapy may increase its levels as well. 20α-Hydroxy-metabolites are probably decreased due to epilepsy itself and not due to the treatment, similarly as cortisol. CBZ probably induces only limited changes leading to a decrease in isopregnanolone, 5α,20α-tetrahydroprogesterone, and androstanediol levels. Concerning the steroids and neuropro-

tection, our results show that the more favorable effect of valproate antiepileptic therapy (in comparison with the carbamazepine one) may be closely associated with the augmented production of neuroprotective C19-steroid metabolites. These primarily originate from the substrates synthesized in adrenal *zona reticularis*. Our data also support the concept that the impairment of gonadal steroidogenesis in MWE is closely associated with the CNS disturbances linked to epilepsy. Therefore, the temporal lobe epilepsy surgery and/or the use of testosterone replacement therapy might further increase the serum levels of neuroprotective GABA-ergic substances like androstanediol and androsterone.

This study is the first attempt to obtain a complex insight on the effects of epilepsy and antiepileptic therapy on the steroid metabolome in adult men. This study enabled to detect the steps of steroid metabolic pathways, which may be influenced by epilepsy and/or affected by antiepileptic therapies. However, further investigations are needed concerning the machinery of these effects.

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References

[1] R. Kancheva, M. Hill, Z. Novak, J. Chrastina, M. Velikova, L. Kancheva, I. Riha, L. Starka, 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 (1–2) (2010) 35–44.
[2] M. Bixo, A. Andersson, B. Winblad, R.H. Purdy, T. Backstrom, Progesterone, 5alpha-pregnane-3,20-dione and 3alpha-hydroxy-5alpha-pregnane-20-one in specific regions of the human female brain in different endocrine states, Brain Res. 764 (1–2) (1997) 173–178.
[3] M. Joels, Steroid hormones and excitability in the mammalian brain, Front. Neuroendocrinol. 18 (1) (1997) 2–48.
[4] J. Pimentel, Current issues on epileptic women, Curr. Pharm. Des. 6 (8) (2000) 865–872.
[5] R. Rupprecht, Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties, Psychoneuroendocrinology 28 (2) (2003) 139–168.
[6] S. Beyenburg, B. Stöffel-Wagner, J. Bauer, M. Watzka, I. Blumcke, F. Bidlingmaier, C.E. Elger, Neuroactive steroids and seizure susceptibility, Epilepsy Res. 44 (2–3) (2001) 141–153.
[7] M.J. Morrell, Epilepsy in women: the science of why it is special, Neurology 53 (4 Suppl. 1) (1999) S42–S48.
[8] H.E. Edwards, W.M. Burnham, A. Mendonca, D.A. Bowlby, N.J. MacLusky, Steroid hormones affect limbic afterdischarge thresholds and kindling rates in adult female rats, Brain Res. 838 (1–2) (1999) 136–150.
[9] H.E. Edwards, W.M. Burnham, N.J. MacLusky, Testosterone and its metabolites affect afterdischarge thresholds and the development of amygdala kindled seizures, Brain Res. 838 (1–2) (1999) 151–157.
[10] P. Wise, Estradiol exerts neuroprotective actions against ischemic brain injury: insights derived from animal models, Endocrine 21 (1) (2003) 11–15.
[11] S. Bastianetto, C. Ramassamy, J. Poirier, R. Quirion, Dehydroepiandrosterone (DHEA) protects hippocampal cells from oxidative stress-induced damage, Brain Res. Mol. Brain Res. 66 (1–2) (1999) 35–41.
[12] X. Mao, S.W. Barger, Neuroprotection by dehydroepiandrosterone-sulfate: role of an NFkappaB-like factor, Neuroreport 9 (4) (1998) 759–763.
[13] P.H. Jellinck, S.J. Lee, B.S. McEwen, Metabolism of dehydroepiandrosterone by rat hippocampal cells in culture: possible role of aromatization and 7-hydroxylation in neuroprotection, J. Steroid Biochem. Mol. Biol. 78 (4) (2001) 313–317.
[14] R. Morfin, L. Starka, Neurosteroid 7-hydroxylation products in the brain, Int. Rev. Neurobiol. 46 (2001) 79–95.
[15] M. Leskiewicz, D. Jantas, B. Budziszewska, W. Lason, Excitatory neurosteroids attenuate apoptotic and excitotoxic cell death in primary cortical neurons, J. Physiol. Pharmacol. 59 (3) (2008) 457–475.
[16] C.E. Weaver Jr., F.S. Wu, T.T. Gibbs, D.H. Farb, Pregnenolone sulfate exacerbates NMDA-induced death of hippocampal neurons, Brain Res. 803 (1–2) (1998) 129–136.
[17] H. Shirakawa, H. Katsuki, T. Kume, S. Kaneko, A. Akaike, Pregnenolone sulphate attenuates AMPA cytotoxicity on rat cortical neurons, Eur. J. Neurosci. 21 (9) (2005) 2329–2335.
[18] P. Klein, A.G. Herzog, Hormonal effects on epilepsy in women, Epilepsia 39 (Suppl. 8) (1998) S9–S16.
[19] C.A. Frye, The neurosteroid 3 alpha, 5 alpha-THP has antiseizure and possible neuroprotective effects in an animal model of epilepsy, Brain Res. 696 (1–2) (1995) 113–120.
[20] D.S. Reddy, Anticonvulsant activity of the testosterone-derived neurosteroid 3alpha-androstanediol, Neuroreport 15 (3) (2004) 515–518.
[21] R.M. Kaminski, H. Marini, W.J. Kim, M.A. Rogawski, Anticonvulsant activity of androsterone and etiocholanolone, Epilepsia 46 (6) (2005) 819–827.
[22] T. Backstrom, A. Andersson, L. Andree, V. Birzniece, M. Bixo, I. Bjorn, D. Haage, M. Isaksson, I.M. Johansson, C. Lindblad, P. Lundgren, S. Nyberg, I.S. Odmark, J. Stromberg, I. Sundstrom-Poromaa, S. Turkmen, G. Wahlstrom, M. Wang, A.C. Wihlback, D. Zhu, E. Zingmark, Pathogenesis in menstrual cycle-linked CNS disorders, Ann. N. Y. Acad. Sci. 1007 (2003) 42–53.
[23] M. Park-Chung, A. Malayev, R.H. Purdy, T.T. Gibbs, D.H. Farb, Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function through distinct sites, Brain Res. 830 (1) (1999) 72–87.
[24] S. Grosso, S. Luisi, R. Mostardini, M. Farnetani, L. Cobellis, G. Morgese, P. Balestri, F. Petraglia, Inter-ictal and post-ictal circulating levels of allopregnanolone, an anticonvulsant metabolite of progesterone, in epileptic children, Epilepsy Res. 54 (1) (2003) 29–34.
[25] R Galli, M. Luisi, C. Pizzanelli, P. Monteleone, E. Casarosa, A. Iudice, L. Murri, Circulating levels of allopregnanolone, an anticonvulsant metabolite of progesterone, in women with partial epilepsy in the postcritical phase, Epilepsia 42 (2) (2001) 216–219.
[26] A.G. Herzog, Hormonal therapies: progesterone, Neurotherapeutics 6 (2) (2009) 383–391.
[27] D.S. Reddy, The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy, Epilepsy Res. 85 (1) (2009) 1–30.
[28] D.S. Reddy, M.A. Rogawski, Neurosteroid replacement therapy for catamenial epilepsy, Neurotherapeutics 6 (2) (2009) 392–401.
[29] H.S. Willenberg, M. Haase, C. Papewalis, M. Schott, W.A. Scherbaum, S.R. Bornstein, Corticotropin-releasing hormone receptor expression on normal and tumorous human adrenocortical cells, Neuroendocrinology 82 (5–6) (2005) 274–281.
[30] L. Ibanez, N. Potau, M.V. Marcos, F. de Zegher, Corticotropin-releasing hormone as adrenal androgen secretagogue, Pediatr. Res. 46 (3) (1999) 351–353.
[31] R. Smith, S. Mesiano, E.C. Chan, S. Brown, R.B. Jaffe, Corticotropin-releasing hormone directly and preferentially stimulates dehydroepiandrosterone sulfate secretion by human fetal adrenal cortical cells, J. Clin. Endocrinol. Metab. 83 (8) (1998) 2916–2920.
[32] S. Mesiano, R.B. Jaffe, Developmental and functional biology of the primate fetal adrenal cortex, Endocr. Rev. 18 (3) (1997) 378–403.
[33] W.E. Rainey, K.S. Rehman, B.R. Carr, The human fetal adrenal: making adrenal androgens for placental estrogens, Semin. Reprod. Med. 22 (4) (2004) 327–336.
[34] M. Brunet, M. Rodamilans, M.J. Martinez-Osaba, J. Santamaria, J. To-Figueras, M. Torra, J. Corbella, F. Rivera, Effects of long-term antiepileptic therapy on the catabolism of testosterone, Pharmacol. Toxicol. 76 (6) (1995) 371–375.
[35] D.S. Reddy, Mass spectrometric assay and physiological–pharmacological activity of androgenic neurosteroids, Neurochem. Int. 52 (4–5) (2008) 541–553.
[36] F. Labrie, A. Belanger, L. Cusan, B. Candas, Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology, J. Clin. Endocrinol. Metab. 82 (8) (1997) 2403–2409.
[37] P. Putignano, G.A. Kaltsas, M.A. Satta, A.B. Grossman, The effects of anticonvulsant drugs on adrenal function, Horm. Metab. Res. 30 (6–7) (1998) 389–397.
[38] M.I. Lossius, E. Tauboll, P. Mowinckel, L. Morkrid, L. Gjerstad, Reversible effects of antiepileptic drugs on reproductive endocrine function in men and women with epilepsy—a prospective randomized double-blind withdrawal study, Epilepsia 48 (10) (2007) 1875–1882.
[39] J.I. Isojarvi, E. Lofgren, K.S. Juntunen, A.J. Pakarinen, M. Paivansalo, I. Rautakorpi, L. Tuomivaara, Effect of epilepsy and antiepileptic drugs on male reproductive health, Neurology 62 (2) (2004) 247–253.
[40] A.G. Herzog, F.W. Drislane, D.L. Schomer, P.B. Pennell, E.B. Bromfield, K.M. Kelly, E.L. Farina, C.A. Frye, Differential effects of antiepileptic drugs on sexual function and reproductive hormones in men with epilepsy: interim analysis of a comparison between lamotrigine and enzyme-inducing antiepileptic drugs, Epilepsia 45 (7) (2004) 764–768.
[41] A.G. Herzog, F.W. Drislane, D.L. Schomer, P.B. Pennell, E.B. Bromfield, B.A. Dworetzky, E.L. Farina, C.A. Frye, Differential effects of antiepileptic drugs on neuroactive steroids in men with epilepsy, Epilepsia 47 (11) (2006) 1945–1948.
[42] L.S. Røste, E. Tauboll, L. Morkrid, T. Bjornenak, E.R. Saetre, T. Morland, L. Gjerstad, Antiepileptic drugs alter reproductive endocrine hormones in men with epilepsy, Eur. J. Neurol. 12 (2) (2005) 118–124.
[43] G.J. Macphee, J.G. Larkin, E. Butler, G.H. Beastall, M.J. Brodie, Circulating hormones and pituitary responsiveness in young epileptic men receiving long-term antiepileptic medication, Epilepsia 29 (4) (1988) 468–475.
[44] J. Rattya, A.J. Pakarinen, M. Knip, M. Repo-Outakoski, V.V. Myllyla, J.I. Isojarvi, Early hormonal changes during valproate or carbamazepine treatment: a 3-month study, Neurology 57 (3) (2001) 440–444.
[45] P. Kwan, F.P. Yip, A.C. Hui, H. Leung, P.W. Ng, K.F. Hui, I.H. Chan, M.H. Chan, C.W. Lam, Effects of valproate or lamotrigine monotherapy on the reproductive endocrine and insulin-related metabolic profile in Chinese adults with epilepsy: a prospective randomized study, Epilepsy Behav. 14 (4) (2009) 610–616.
[46] J. Rattya, J. Turkka, A.J. Pakarinen, M. Knip, M.A. Kotila, O. Lukkarinen, V.V. Myllyla, J.I. Isojarvi, Reproductive effects of valproate, carbamazepine, and oxcarbazepine in men with epilepsy, Neurology 56 (1) (2001) 31–36.
[47] L.J Stephen, P. Kwan, D. Shapiro, M. Dominiczak, M.J. Brodie, Hormone profiles in young adults with epilepsy treated with sodium valproate or lamotrigine monotherapy, Epilepsia 42 (8) (2001) 1002–1006.

[48] B. Stöffel-Wagner, J. Bauer, D. Flugel, W. Brennemann, D. Klingmuller, C.E. Elger, Serum sex hormones are altered in patients with chronic temporal lobe epilepsy receiving anticonvulsant medication, Epilepsia 39 (11) (1998) 1164–1173.
[49] S. Svalheim, E. Tauboll, G. Luef, A. Lossius, M. Rauchenzauner, F. Sandvand, M. Bertelsen, L. Morkrid, L. Gjerstad, Differential effects of levetiracetam, carbamazepine, and lamotrigine on reproductive endocrine function in adults, Epilepsy Behav. 16 (2) (2009) 281–287.
[50] J. Bauer, B. Stöffel-Wagner, D. Flugel, M. Kluge, J. Schramm, F. Bidlingmaier, C.E. Elger, Serum androgens return to normal after temporal lobe epilepsy surgery in men, Neurology 55 (6) (2000) 820–824.
[51] K. Mikkonen, P. Tapanainen, A.J. Pakarinen, M. Paivansalo, J.I. Isojarvi, L.K. Vainionpaa, Serum androgen levels and testicular structure during pubertal maturation in male subjects with epilepsy, Epilepsia 45 (7) (2004) 769–776.
[52] M. Hill, A. Parizek, R. Kanceva, M. Duskova, M. Velikova, L. Kriz, M. Klimkova, A. Paskova, Z. Zizka, P. Matucha, M. Meloun, L. Starka, 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. (2010).
[53] M. Hill, R. Hampl, D. Lukac, O. Lapcik, V. Pouzar, J. Sulcova, Elimination of cross-reactivity by addition of an excess of cross-reactant for radioimmunoassay of 17alpha-hydroxypregnenolone, Steroids 64 (5) (1999) 341–355.
[54] J. Trygg, E. Holmes, T. Lundstedt, Chemometrics in metabonomics, J. Proteome Res. 6 (2) (2007) 469–479.
[55] J. Trygg, S. Wold, Orthogonal projections to latent structure, J. Chemometrics 16 (2002) 119–128.
[56] G. Murialdo, C.A. Galimberti, S. Fonzi, R. Manni, P. Costelli, C. Parodi, F. Torre, G.P. Solinas, A. Polleri, A. Tartara, Sex hormones, gonadotropins and prolactin in male epileptic subjects in remission: role of the epileptic syndrome and of antiepileptic drugs, Neuropsychobiology 30 (1) (1994) 29–36.
[57] J.I Isojarvi, M. Repo, A.J. Pakarinen, O. Lukkarinen, V.V. Myllyla, Carbamazepine, phenytoin, sex hormones, and sexual function in men with epilepsy, Epilepsia 36 (4) (1995) 366–370.
[58] L. Starka, H. Pospisilova, M. Hill, Free testosterone and free dihydrotestosterone throughout the life span of men, J. Steroid Biochem. Mol. Biol. 116 (1–2) (2009) 118–120.
[59] F. Labrie, V. Luu-The, S.X. Lin, J. Simard, C. Labrie, M. El-Alfy, G. Pelletier, A. Belanger, Intracrinology: role of the family of 17 beta-hydroxysteroid dehydrogenases in human physiology and disease, J. Mol. Endocrinol. 25 (1) (2000) 1–16.
[60] A.G. Herzog, Psychoneuroendocrine aspects of temporolimbic epilepsy. Part I. Brain, reproductive steroids, and emotions, Psychosomatics 40 (2) (1999) 95–101.
[61] A.G. Herzog, Psychoneuroendocrine aspects of temporolimbic epilepsy. Part II: epilepsy and reproductive steroids, Psychosomatics 40 (2) (1999) 102–108.
[62] S. Duncan, J. Blacklaw, G.H. Beastall, M.J. Brodie, Antiepileptic drug therapy and sexual function in men with epilepsy, Epilepsia 40 (2) (1999) 197–204.
[63] E. Motta, D. Rosciszewska, B. Buntner, Serum ACTH levels in patients treated for epilepsy, Neurol. Neurochir. Pol. 28 (3) (1994) 317–323.
[64] P.A. Lapchak, D.F. Chapman, S.Y. Nunez, J.A. Zivin, Dehydroepiandrosterone sulfate is neuroprotective in a reversible spinal cord ischemia model: possible involvement of GABA(A) receptors, Stroke 31 (8) (2000) 1953–1956, discussion 1957.
[65] A. Malayev, T.T. Gibbs, D.H. Farb, Inhibition of the NMDA response by pregnenolone sulphate reveals subtype selective modulation of NMDA receptors by sulphated steroids, Br. J. Pharmacol. 135 (4) (2002) 901–909.
[66] M. Park-Chung, F.S. Wu, D.H. Farb, 3 alpha-Hydroxy-5 beta-pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons, Mol. Pharmacol. 46 (1) (1994) 146–150.
[67] M.B. Nicol, J.J. Hirst, D. Walker, Effects of pregnanolone on behavioural parameters and the responses to GABA(A) receptor antagonists in the late gestation fetal sheep, Neuropharmacology 38 (1) (1999) 49–63.