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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-