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Determination of steroid metabolome as a possible tool for laboratory diagnosis of schizophrenia

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ABSTRACT

Metabolomic studies represent a promising tool for early diagnosis of schizophrenia. The aim of this study was to find differences in the steroid spectrum in patients and controls, and to assess the diagnosis of schizophrenia by building a predictive model based on steroid data.

Thirty-nine serum steroids (22 neuroactive steroids and their metabolites and 17 polar conjugates) representing steroid metabolome were measured by gas chromatography–mass spectrometry in 22 drug-naive (first episode) schizophrenia patients (13 men and 9 women) before and after six-month treatment with atypical antipsychotics. The results were compared to the data from healthy subjects (22 males, 25 females). In summary the following significant differences were found: (1) In both sexes higher levels of pregnenolone sulfate and sulfated 5α- as well as 5β-saturated metabolites of C21-steroids in progesterone metabolic pathway were found in patients, pointing to decreased activity of sulfatase. (2) In a few instances decreased levels of the respective 5α-metabolites of C21 steroids were found in patients. (3) As C19 steroids concern, in both sexes there were considerably lowered levels of 5β-reduced metabolites in patients. On the other hand, with only a few exceptions, the treatment did not significantly influence most steroid levels. Further, to assess the relationships between schizophrenia status and steroid levels and to build the predictive model of schizophrenia, multivariate regression with reduction of dimensionality (the method of orthogonal projections to latent structures, OPLS) was applied. Irrespective of the small number of patients, use of this model enabled us to state the diagnosis of schizophrenia with almost 100% sensitivity.

Our findings suggest that the assessment of steroid levels may become a valid and accurate laboratory test in psychiatry. A limitation of our study is the absence of subjects with a diagnosis other than schizophrenia, so we cannot conclude whether the results are specific for schizophrenia. On the other hand, steroid metabolome model may be used as a diagnostic tool for further studies.

1. Introduction

Neuroactive steroids generally affect nervous cells in a non-genomic way [1]. Their role in the brain physiology and pathophysiology consists mostly in their ability to modulate (positively or negatively) ligand-gated ion channels as the type A γ-aminobutyric-acid receptors ($GABA_A$-R) or glutamate, especially N-methyl D-aspartate receptors (NMDA-R), and also the σ_1 receptor. Positive modulation of $GABA_A$-R is pharmacologically manifested as benzodiazepine-like inhibition with anxiolytic, analgesic, anticonvulsant and hypnotic effects. NMDA-R serves as a calcium channel, the positive modulation of which results in increase of Ca^{2+} into neuronal cells with excitatory effects. The neuromodulating effect of neuroactive steroids depends on their chemical structure (for reviews see [2–6]).

Human endogenous C21 steroids acting as positive modulators of $GABA_A$ receptors include (1) saturated metabolites in progesterone metabolic pathway, such as allopregnanolone (3α-hydroxy-5α-pregnan-20-one, 3α,5α-tetrahydroprogesterone, 3α,5α-THP), pregnanolone (3α-hydroxy-5β-pregnan-20-one, 3α,5β-tetrahydroprogesterone, 3α,5β-THP) and corresponding 21-hydroxyderivatives [7,8] and (2) some C19 steroids, such as androsterone (3α-hydroxy-5α-androstan-17-one), etiocholanolone (3α-hydroxy-5β-androstan-17-one) and androstanediol (5α-androstane-3α,17β-diol) [2,3,9–11]. On the other hand, the sulfates of all pregnanolone isomers are negative modulators of $GABA_A$ – with excitatory effects [12]. In addition, sulfates of 5α-pregnanolone isomers and unsaturated 5-ene-steroids as pregnenolone sulfate and dehydroepiandrosterone sulfate (DHEAS) act as positive NMDA-R modulators

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with excitatory effect [4], while the sulfates of 5β-pregnanolone isomers are inhibitory [4,13–15]. Gonadal sex steroids also belong to modulators of GABA and NMDA receptors [16].

In the past decade attention also turned to σ_1 and σ_2 receptors and their ligands, and the way of their implication in neuroprotection and neuronal plasticity. When activated by their ligands, σ_1 receptors may function as chaperons modulating the NMDA receptor via protein-protein interaction [17]. Therefore, σ_1 receptors may indirectly regulate calcium flux into the neurons [17,18]. Dehydroepiandrosterone (DHEA) and other neurosteroids with antidepressant activity can bind directly to the σ_1 receptors [17,19,20]. Neurosteroids may thus serve as one of the connecting links between glutamate (NMDA) receptors and σ_1 receptors. Hormonal steroids, especially estrogens are also modultoras of dopamine receptor, dopamine transport and metabolism [21].

The past two decades have also supplied much evidence that alterations in circulating levels of neuroactive steroids are associated with pathological processes in the central nervous system [6–8,22]. Recent studies have shown that patients' concentrations of some circulating neuroactive steroids, mainly those synthesized in the brain (neurosteroids), are changed in comparison with age-matched healthy controls [6–8,23]. Preclinical studies, focused mostly on the levels of neurosteroids in mental disorders, however often yielded contradictory results and thus their role in the etiology of the pathogenic processes remain unclear.

Our recent results indicate that several GABAergic steroid metabolites may be altered in schizophrenic men [24]. In general, the whole steroid metabolome as a complex of steroids present in blood may be changed in schizophrenia patients. Such a metabolome has not yet been studied in its complexity. The aim of this work is to compare the steroid metabolome in the group of first-episode schizophrenia patients with sex- and age-matched healthy subjects, and to find out how the metabolome is affected by the treatment with atypical antipsychotics, namely whether the treatment might reinstate steroid levels to values commonly found in healthy subjects. The further task was an attempt to build a predictive model of schizophrenia based on the laboratory determination of steroid metabolome.

2. Methods

2.1. Patients and controls

The patient group consisted of 13 males aged 22–52 years (median 31 years) and 8 females aged 24–52 (median 35 years) with their first episode of schizophrenia. Diagnosis of schizophrenia was made according to the Structured Clinical Interview for DSM-IV: dg. paranoid schizophrenia (F 20.0, $n = 15$), undifferentiated schizophrenia (F 20.3, $n = 6$). The treatment with atypical antipsychotics was initiated and maintained for the next 6 months. Patients were treated with olanzapine or "non-olanzapine" type of antipsychotic drugs as follows: men, olanzapine $n = 6$, mean daily dose 10–15 mg/day, and non-olanzapine, $n = 7$, 3–4 mg/day of risperidone ($n = 5$), and 300 mg/day of amisulpride ($n = 2$); women: olanzapine $n = 4$, 15 mg/day, non-olanzapine n = 5, 4 mg/day of risperidone ($n = 4$), and amisulpride 300 mg/day ($n = 1$). Open-label antipsychotic treatment was prescribed in a flexible dosing schedule, adjusted according to the treating physician's discretion. The non-steroidal laboratory data of the same group of schizophrenic men were reported elsewhere. They included basic parameters of thyroid function, fasting glucose, basic lipid parameters, prolactin and four thiols [25]. The patients whose medication had to be changed during the trial were excluded from the study. The control group consisted of 19 healthy males aged 23–53 years (median 35 years), and 13 women aged 23–51 years (median 35 years), with no personal or family history of psychiatric illness. All control subjects were checked for absence of metabolic disorders as evaluated by determination of fasting glycemia, lipid spectrum and also basic parameters of thyroid function (TSH, fT4) and did not use any medication. All women, patients as well as controls, were in the follicular phase of their menstrual cycle at the time of blood collection. The capacity of subjects to provide consent was determined by their legal status and additionally assessed by their treating physicians. None of the participants was judged as having reduced ability to provide consent, nor had a legally restricted capacity that would require their obtaining consent by proxy. The study was approved by the Local Ethical Committee of both involved institutions (Prague Psychiatric Center and the Institute of Endocrinology, Prague).

2.2. Blood collection

Blood was collected at 8:00 after overnight fasting. No medication was used before the first blood collection. The next examination was completed following 6 months of steady treatment by atypical antipsychotic medication.

2.3. Analysis of circulating steroids

Twenty-two unconjugated steroids and 17 steroid polar conjugates (sulfates and glucuronides) were assessed in the serum of patients and controls by the gas chromatographic–mass spectrometric analysis described elsewhere [26]. Only steroids detectable by the above method were measured so that only 17 unconjugated steroids and 14 steroid polar conjugates were quantified in men. The list of trivial and systematic names of steroids and their abbreviations is given in Appendix A.

The steroid standards were purchased from Steraloids (Newport, RI, USA), the Sylon B from Supelco (Bellefonte, PA, USA), the methylamine hydrochloride from Sigma (St. Louis, MO, USA) and the solvents from Merck (Darmstadt, Germany).

2.4. Statistical analysis

The differences between the groups of healthy controls, untreated patients and patients after treatment with atypical antipsychotics were evaluated separately for each sex using the age-adjusted ANCOVA followed by Least Significant Difference multiple comparisons ($p = 0.05$). The age adjustment was applied to separate the variability between groups from the variability explained by age and to eliminate the potential effect of age differences between control group and patient groups in men.

The multivariate regression with reduction of dimensionality (the method of orthogonal projections to latent structures, OPLS) was applied to assess the relationships between schizophrenia status, age and steroid levels and to build the models for prediction of schizophrenia from the values of circulating steroids. The models were constructed separately for men and women. The relevant variables, the levels of measured steroids, shown in Tables 3 and 4 (see Section 3) have been chosen by using variable importance ("VIP") statistics. In all instances the software SIMCA, version 12.1.1.1. (UNIMETRICS, Umea, Sweden) was used.

3. Results

The levels of C21 and C19 steroids, constitutes of steroid metabolome in the groups of healthy men, untreated male patients and patients after 6-month treatment with atypical antipsychotics, are shown in Tables 1a (unconjugated steroids) and 1b (steroid conjugates). The results from analogous groups of women are