Journal of Steroid Biochemistry & Molecular Biology 119 (2010) 35–44

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Peripheral neuroactive steroids may be as good as the steroids in the cerebrospinal fluid for the diagnostics of CNS disturbances

Radmila Kancheva[a,*], Martin Hill[a], Zdeněk Novák[b], Jan Chrastina[b], Marta Velíková[a], Lyudmila Kancheva[a], Ivo Říha[b], Luboslav Stárka[a]

[a] Institute of Endocrinology, Národní třída 8, Prague CZ 116 94, Czech Republic
[b] St. Anne's University Hospital, Pekařská 53, 656 91 Brno, Czech Republic

ARTICLE INFO

Article history:
Received 3 July 2009
Received in revised form 4 December 2009
Accepted 8 December 2009

Keywords:
Steroids
Cerebrospinal fluid (CSF)
Plasma
Metabolome
GC–MS

ABSTRACT

To compare the predictivity of the neuroactive steroids in the cerebrospinal fluid and peripheral blood for the diagnostics of CNS disturbances, eighteen unconjugated steroids were quantified in the cerebrospinal fluid (CSF) from the 3rd ventricle and 18 unconjugated steroids and 7 steroid polar conjugates were measured in the serum using GC–MS and RIA. Eight postmenopausal women (56–78 years of age) and 7 men (22–88 years of age) with hydrocephalus were enrolled in the study. The sensitivity of the method ranged from low femtogram to low picogram levels depending on the steroid fragmentation pattern. Using multivariate regression, a model for simultaneous prediction of the CSF steroids from the serum steroids was completed. Then, the penetrability of the individual steroids across the blood-brain-barrier was evaluated and the sources of various brain steroids were estimated. Our data show that a part of the steroids may be synthesized de novo in the CNS. However, substantial part of the steroid metabolites may be synthesized in the CNS from the steroid precursors or directly transported from the periphery. The CNS in situ synthesis and transport from periphery might be complementary in some cases, i.e. brain synthesis might provide minimum level of steroids, which are indispensable for the CNS functions.

1. Introduction

Although several authors [1–4] have analyzed the steroid transport between the periphery and the brain, the respective data is still incomplete. Hence, we evaluated the correlations of the steroids between the periphery and the cerebrospinal fluid (CSF), considering also the effect of the steroid conjugation in the periphery. In addition, we have followed the correlations between the steroids within the CSF. The aim of the study was to estimate to what extent the peripheral steroids may contribute to the steroid metabolome in the CNS and how important may be the contribution of the *in situ* brain synthesis. To our concept the steroid brain *in situ* synthesis provides minimum amount of steroids, which is indispensable for the CNS functions in the situations when the peripheral sources misfire. However, usually in physiological situations the penetration of peripheral steroids across the blood-brain-barrier provides the majority of the CNS steroids, at least in the form of the parent substances, which are further metabolized in the CNS. We also believe that changes in the peripheral steroid production are promptly reflected in the CNS steroid metabolome. Therefore, the predictive value of the peripheral steroids and particularly the neuroactive steroids could be comparable with the predictivity of the CSF steroids. Hence, the substantially less invasive collection of the peripheral steroids may be as good as the collection of the CSF ones for the diagnostics of CNS diseases, which are most probably connected with an imbalance in peripheral steroidogenesis (like postpartum depressions, catamenial epilepsy, premenstrual syndrome and possibly the affective disorders) and could also reflect the sex differences and various physiological changes like pregnancy, parturition or aging.

For the verification of these assumptions we evaluated the correlations between peripheral and CSF steroids using a wide spectrum of bioactive steroids, their precursors and metabolites. The variety of steroids measured in the present study is unique.

2. Materials and methods

2.1. Subjects

Eight postmenopausal women (56–78 years) and 7 men (22–88 years) underwent an endoscopic 3rd ventriculostomy because of obstructive hydrocephalus.

* Corresponding author. Tel.: +420 2 24905 238; fax: +420 2 24905 325.
E-mail addresses: rkanceva@endo.cz (R. Kancheva), mhill@endo.cz (M. Hill), zdenek.novak@fnusa.cz (Z. Novák), jan.chrastina@fnusa.cz (J. Chrastina), mvelikova@endo.cz (M. Velíková), lkancheva@endo.cz (L. Kancheva), ivo.riha@fnusa.cz (I. Říha), lstarka@endo.cz (L. Stárka).

0960-0760/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2009.12.006

Table 1
List of steroids under investigation and the methods used for quantification.

1	A2. . .androstenedione (GC–MS)
2	A3α5α. . .5α-androstane-3α-hydroxy-17-one; androsterone (GC–MS)
3	A3β5α. . .5α-androstane-3β-hydroxy-17-one; epiandrosterone (GC–MS)
4	AT7α. . .5-androstene-3β,7α,17β-triol (GC–MS)
5	AT7β. . .5-androstene-3β,7β,17β-triol (GC–MS)
6	Cort. . .cortisol (RIA)
7	DHEA. . .dehydroepiandrosterone (GC–MS)
8	DHEA16α. . .3β,16α-dihydroxy-5-androstene-17-one; 16α-hydroxy-DHEA (GC–MS)
9	DHEA7α. . .3β,7α-dihydroxy-5-androstene-17-one; 7α-hydroxy-DHEA (GC–MS)
10	DHEA7β. . .3β,7β-dihydroxy-5-androstene-17-one; 7β-hydroxy-DHEA (GC–MS)
11	P3α5α. . .3α-hydroxy-5α-pregnane-20-one; allopregnanolone (GC–MS)
12	P3β5α. . .3β-hydroxy-5α-pregnane-20-one; isopregnanolone; epiallopregnanolone (GC–MS)
13	Preg. . .pregnenolone (GC–MS)
14	Preg16α. . .3β,16α-dihydroxy-5-pregnene-20-one; 16α-hydroxy-pregnenolone (GC–MS)
15	Prog. . .progesterone (RIA)
16	Prog16α. . .16α-hydroxy-4-pregnene-3,20-dione; 16α-hydroxy-progesterone (GC–MS)
17	Prog17. . .17-hydroxy-progesterone (RIA)
18	T. . .testosterone (GC–MS)

C at the end of the abbreviation, polar conjugates of the steroid; GC–MS, gas chromatography–mass spectrometry; RIA, radioimmunoassay.

2.2. Sample collection

The surgeries were performed under a general endotracheal anesthesia in patients who were treated in the Department of Neurosurgery of the Faculty Hospital St. Anne's in Brno. The patients were operated for either tumorous or non-tumorous lesions. Neuroendoscopic system Wolf or Storz was used for the surgery. Neuroendoscopic access to the third ventricle [5,6] was as follows: At the beginning of the neuroendoscopic procedure samples of the cerebrospinal fluid (CSF) were collected from 3rd ventricle through the foramen of Monro and from the lateral ventricle afterwards for cytological analysis, for tumor markers and steroid analysis. Particular attention was paid not to dilute the samples and biopsy catheter of own construction was used for the sampling. Before the surgery a peripheral blood sample (10 mL) was taken from the cubital vein. The blood components were separated and the serum and CSF samples were subsequently stored in deep freeze at −80 °C until analyzed.

2.3. Steroid analyses

Eighteen unconjugated steroids were quantified in the cerebrospinal fluid from the 3rd ventricle (CSF) and 18 unconjugated steroids and 7 steroid polar conjugates were measured in the serum using GC–MS and RIA (Table 1).

2.4. Chemicals and reagents

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

2.5. Instruments

The GC–MS system was supplied by Shimadzu (Kyoto, Japan). The GCMS-QP2010 Plus system consisted of a gas chromatograph equipped with automatic flow control, AOC-20s and an autosampler. Quadrupole detector with electron-impact ionization was used for the analyses. Adjustable electron voltage of 10–195 V was set to 70 V. Emission current was set to 160 μA. A capillary column with a medium polarity RESTEK Rxi (diameter 0.25 mm, length 15 m, film thickness 0.1 μm was used for analyses. The temperature of the injection port, ion source and interface was maintained at 220, 300, and 310 °C, respectively. Analyses were carried out in the splitless mode with a constant linear velocity of the carrier gas (He), which was maintained at 60 cm/s. The septum purge flow was set up to 3 mL/min. The samples were injected using the high-pressure mode which was applied at 200 kPa and this pressure was maintained for 1 min. The detector voltage was set to 1.4 kV.

2.6. Sample preparation for GC–MS analysis

When using the GC–MS platform, the unconjugated steroids were extracted from 1 mL of plasma or CSF with diethyl-ether (3 mL). The diethyl-ether extract was dried in the block heater at 37 °C. The lipids in the dry residue of the diethyl-ether extract were separated by partitioning between a mixture of methanol-water 4:1 (1 mL) and pentane (1 mL). The pentane phase was discarded and the polar phase was dried in the vacuum centrifuge at 60 °C (2 h). The dry residue from the polar phase was derivatized first with 50 μL methoxylamine-hydrochloride solution in pyridine (2%) on oxo-groups (60 °C, 1 h). The mixture after the first derivatization was dried in the flow of nitrogen and the dry residue was treated with 50 μL reagent Sylon B (99% of bis(trimethylsilyl)-trifluoroacetamide and 1% of trimethylchlorosilane) forming trimethylsilyl derivatives on hydroxy-groups (TMS-MOX derivatives) (90 °C, 1 h). Finally, the mixture after the second derivatization step was dried in the flow of nitrogen, the dry residue was dissolved in 20 μL of isooctane and 1 μL of the solution was used for GC–MS analysis.

The steroid conjugates remaining in the polar residues after diethyl-ether extraction were analyzed as follows: the polar residues were dried in the vacuum centrifuge at 37 °C (5 h) and the dry residues containing steroid polar conjugates (mostly sulfates and glucuronides) were hydrolyzed as described elsewhere [7]. The hydrolyzed samples were again dried in the vacuum centrifuge at 37 °C (5 h). The dried residues were reconstituted with 1 mL of chromatographic water and then extracted from 1 mL of water solution into 3 mL diethyl-ether. The diethyl-ether extract was further processed in the same way as the free steroids. In contrast to the sample preparation of free steroids, the dry residue after the second derivatization step was dissolved in 200 μL of isooctane instead of the 20 μL of isooctane. Prior to further processing, the original samples and the polar phases after diethyl-ether extraction (which were used for the quantification of the steroid conjugates) were spiked with 17α-estradiol (as an internal standard) to attain a concentration of 1 and 10 ng/mL, respectively. The internal standard was recorded at effective masses m/z = 231, 285 and 416. The addition of the internal standard to the body fluid before sample preparation (free steroids) and to polar phase after diethyl-ether extraction (conjugated steroids) assured that the losses during the sample processing were not critical for the steroid quantification.

2.7. Temperature and pressure gradients for the GC–MS analysis of trimethylsilyl derivatives and the retention times of the steroids

To effectively utilize the biological material and to suitably separate the isomers with similar fragmentation, the individual samples were applied in independent courses, in each case employing a part of the steroids under investigation. The choices of the steroids measured within the individual courses, the temperature and pressure gradients, and the effective masses used for the measurement in selected ion monitoring mode were all optimized to attain a min-