ORIGINAL ARTICLE

7-Hydroxylated derivatives of dehydroepiandrosterone in the human ventricular cerebrospinal fluid

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Abstract

OBJECTIVE: Dehydroepiandrosterone is a long established neuroactive steroid. Some authors documented that 7-oxygenated derivatives of this steroid may be responsive at least by part for its physiological activity.
METHODS: In the ventricular cerebrospinal fluid obtained from 15 patients with hydrocephalus (8 postmenopausal women and 7 men) potentially neuroactive steroid 7-oxygenated derivatives of dehydroepiandrosterone were quantified using gas chromatography/mass spectrometry.
RESULTS: Besides free dehydroepiandrosterone 7-oxygenated steroids such as 7α- and 7β-hydroxy-dehydroepiandrosterone, 5-androstene-3β,7α,17β-triol and 5-androstene-3β,7β,17β-triol in picomolar concentration in serum and cerebrospinal fluid were found.
CONCLUSION: Dehydroepiandrosterone and its 7-oxygenated derivatives are present in ventricular cerebrospinal fluid in concentration 2 –100 times lower than in serum.

INTRODUCTION

7α-hydroxy-dehydroepiandrosterone was first isolated and identified by Okada (Okada *et al.* 1959) in urine of a patient with adrenal carcinoma and soon thereafter by one of us (Stárka 1961, Stárka *et al.* 1962) in the urine of normal men and women. 7-hydroxylation in rat liver homogenate microsomes was characterized (Stárka nad Kutová 1962) and the 7-hydroxylation of dehydroepiandrosterone was discovered in various tissues, including brain (Akwa *et al.* 1992, 1993, Doostzadeh & Morfin 1996, Doostzadeh *et al.* 1997, Rose *et al.* 1997). Numerous authors paid attention to the presence and role of 7-oxygeneted dehydroepiandrosterone derivatives (for review see Morfin & Starka 2001) in the brain.

At the cellular level, irreversible 7α-hydroxylation of 3β-hydroxy-5-ene steroids produces derivatives, which exert anti-glucocorticoid, immunity-promoting, and protective activities. In the brain, as „neuroprotective steroids" and immunity promoters, 7α-hydroxysteroids could contribute to the panels of cellular protection and defense

Whereas human brain tissue in vivo is only exceptionally accessible to the analytical determination of stored steroids, we aimed at least to describe the occurrence 7-oxygenated steroids in

the ventricular cerebrospinal fluid and to compare their concentration in the fluid and in circulating serum in the periphery.

Neuroendoscopy has achieved extensive acceptance among neurosurgeons as a minimally invasive technique for the treatment of patients affected by blocked hydrocephalus. During endoscopic procedures minimal CSF amounts from selected anatomic sites of the ventricles can be withdrawn. Steerable endoscopes are used and their flexibility facilitates the aspiration of CSF during the preliminary inspection through the ventricular cavities, without any interference with the surgical actions or additional risks for the patients. In this preliminary study the concentrations of dehydroepiandrosterone and related 7-oxygenated metabolites in the third ventricle were examined.

AIM OF THE STUDY

The goals were to determine in serum and cerebrospinal fluid (CSF) from the 3rd ventricle the levels of free dehydroepiandrosterone (DHEA) and its 7-hydroxylated derivatives: 7α-hydroxy-dehydroepiandrosterone (DHEA7α), 7β-hydroxy-dehydroepiandrosterone (DHEA7β), 5-androstene-3β,7α,17β-triol (AT7α) and 5-androstene-3β,7β,17β-triol (AT7β) and to show to what extent differs their ratio of concentrations in serum and CSF in postmenopausal women and men.

SUBJECTS AND MATERIALS

Patients

Patients – 8 postmenopausal women and 7 men – underwent the endoscopic 3rd ventriculostomy (ETV) for obstructive hydrocephalus. All surgeries were performed under general endotracheal anesthesia in patients treated in Dept. of Neurosurgery MF MU FH St. Ann, 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 third ventricle (Longatti *et al.* 2004, Hellwig *et al.* 2005) was as follows: At the beginning of the neuroendoscopic procedure samples of cerebrospinal fluid (CSF) were collected from 3rd ventricle through 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 sample and biopsy catheter of own construction was used for the sampling. Before surgery peripheral blood sample (10 ml) was taken from the cubital vein. The blood components were separated and serum and CSF samples were subsequently stored in deep freeze at –80° C until worked up in the laboratory.

Sample collection

Cooled plastic tubes were used for blood and CSF sampling. Serum was obtained after centrifugation for 5 minutes at 2000 g at 0°C. Serum samples were stored at -20°C until analyzed.

Steroids and chemicals

The steroids were from Steraloids (Wilton, NH, USA). The solvents for the extraction and HPLC, were of an analytical grade, from Merck (Darmstadt, Germany). The derivatization agent Sylon BFT was purchased from Supelco (Bellefonte, PA, USA).

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 autosampler and a quadrupole electron-impact detector with an adjustable electron voltage of 10–195 V. 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.

Steroid analysis

The levels of steroids listed in the Table 1 were measured in the maternal and fetal body fluids using GC-MS. The unconjugated steroids were extracted from 1 ml of serum or cerebrospinal fluid 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 hours). The dry residue from the polar phase was derivatized first with methoxylamine-hydrochloride solution in pyridine (2 %) on oxo-groups (60°C, 1 hour). The mixture after the first derivatization was dried in the flow of nitrogen and the dry residue was treated with the reagent Sylon B (99% of bis(trimethylsilyl)-trifluoroacetamide and 1% of trimethylchlorosilane) forming trimethylsilyl derivatives on hydroxy-groups (TMS-MOX derivatives) (90° C, 1 hour). 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.

Prior to further processing, the original samples were spiked with 17α-estradiol (as an internal standard) to attain a concentration of 1ng/ml and 10 ng/ml, respectively. The internal standard was recorded at effective masses m/z = 285 and 416. The addition of internal standard to body fluid before sample preparation assured that the losses during the sample processing were not critical for steroid quantification.

Table 1. Steroids included in the study

Nomenclature according to IUPAC	Trivial name	Abbreviation used
3β-hydroxy-5-androsten-17-one	dehydroepiandrosterone (DHEA)	DHEA
3β,7α-dihydroxy-5-androsten-17-one	7α-hydroxy-dehydroepiandrosterone	DHEA7α
3β,7β-dihydroxy-5-androsten-17-one	7β-hydroxy-dehydroepiandrosterone	DHEA7β
5-androstene-3β,7α,17β-triol	androstenetriol-7α	AT7α
5-androstene-3β,7β,17β-triol	androstenetriol-7β	AT7β

Table 2. Conditions for the gas-chromatographic separation of steroids

Method	Conditions: initial conditions (final temperature, temperature gradient, hold time) (°C, °C·min^{-1}, min)				Initial pressure (kPa)	Injection temp. (°C)	Overall time (min)
step:	1	2	3	4			
G1	80 (–, 1)	190 (40, 0)	210 (4, 0)	300 (20, 5)	34	220	18.25
G2	81 (–, 1)	200 (40, 0)	240 (8, 0)	300 (40, 5)	34	220	15.5

Temperature gradients used for steroid analysis at constant linear velocity 60 cm·s^{-1}

Table 3. Data for GC/MS analysis of DHEA and its 7α/β-hydroxyderivatives

Gradient	Steroid	m/z (Da)	Retention time (min) peak1	σ
G1	Dehydroepiandrosterone	268, 358	10.3	0.001
G2	7α-Hydroxy-dehydroepiandrosterone	266, 356, 387	7.96	0.011
G2	7β-Hydroxydehydroepiandrosterone	266, 356, 387	8.82	0.012
G1	Androstene-3β,7α,17β-triol	208, 327, 432	8.41	0.016
G1	Androstene-3β,7β,17β-triol	208, 327, 432	9.72	0.012

σ … standard deviation of retention time

Instrument setup

Electron-impact ionization was used for the analyses. Electron voltage was set up to 70 V and emission current to 160 μA. The temperature of the ion source and interface were maintained at 260°C and 310°C, respectively. Analyses were carried out 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. Samples were injected using the on-column injection mode. The detector voltage was set to 1.4 kV

Temperature and pressure gradients for the GC-MS analysis of steroids after derivatization and the retention times of the steroids

To effectively utilize the biological material, the individual samples were applied in three 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 (SIM) mode were all optimized to attain minimum limit of detection (LOD) at sufficient selectivity. The temperatures and pressure gradients for the detection of steroids are shown in Table 2. The effective masses, retention times of chromatographic peaks, sequence number of injection for steroid groups and gradients that were used for quantification of individual steroids are shown in Table 3. In all cases, the mixtures of authentic standards were processed in the same way as samples. The mixtures were specific for each of the independent courses as mentioned above. The standards were injected in three different amounts for each steroid (10, 100 and 1000 pg).

For evaluation of linearity, increasing volumes of the mixtures of pooled maternal serum with water for chromatography (300+700, 400+600, 500+500, 600+400, 700+300, 800+200, 900+100 and 1000+0 ml) were assayed. The two-parameter linear regression was used for evaluation of the relationships between peak areas and volume of the serum.

Table 4. Concentrations (nmol/l) of the free steroids in CSF and free and conjugated steroids in serum

Steroid	CSF from the 3rd ventricle				Serum, free steroids				Serum, conjugated steroids			
	n	Median	Quartiles		n	Median	Quartiles		n	Median	Quartiles	
			lower	upper			lower	upper			lower	upper
DHEA	15	0.078	0.058	0.154	13	2.28	1.11	4.47	11	1544	726	2095
DHEA7α	15	0.300	0.178	0.375	13	0.79	0.52	1.28	12	4.82	2.24	9.64
DHEA7β	15	0.0369	0.0107	0.0538	13	0.314	0.171	0.442	12	2.07	1.37	6.06
AT7α	15	0.0068	0.0038	0.0177	13	0.095	0.040	0.167	11	0.86	0.27	2.79
AT7β	15	0.0119	0.0035	0.0205	13	0.0504	0.0339	0.0829	11	0.95	0.39	4.28

Table 5. Ratio of concentrations of the steroids in ventricular cerebrospinal fluid and serum

Steroid	Serum, free/ CSF				Serum, conjugates/ CSF				Serum, conjugates/ serum, free			
	n	median	Quartiles		n	median	Quartiles		n	median	Quartiles	
			lower	upper			lower	upper			lower	upper
DHEA	13	23.3	14.8	45.8	11	11291	6490	24607	11	482	302	911
DHEA7α	13	3.27	2.61	3.69	12	17.3	9.1	44.9	12	5.7	2.5	19
DHEA7β	13	10.2	7.7	11.2	12	134	44	328	12	9.4	6.5	35.3
AT7α	13	9.6	5.9	11.2	11	95	26	252	11	8.4	2.3	51.9
AT7β	13	6.26	3.94	8.3	11	113	24	341	11	10	6	102

Table 6. Pearson's correlation of the concentrations ratios of the steroids

Steroid	CSF vs. serum free steroids			CSF vs. serum conjugates			Serum free steroids vs. serum conjugates		
	r	p	n	r	p	n	r	p	n
DHEA	**0.820**	**<0.001**	**13**	0.532	0.092	11	**0.620**	**0.042**	**11**
DHEA7α	**0.917**	**<0.001**	**13**	0.049	0.879	12	–0.044	0.892	12
DHEA7β	**0.941**	**<0.001**	**13**	0.360	0.250	12	0.208	0.516	12
AT7α	**0.868**	**<0.001**	**13**	0.003	0.994	11	–0.302	0.366	11
AT7β	**0.890**	**<0.001**	**13**	–0.294	0.380	11	–0.513	0.107	11

Pearson's correlation after data transformation for attaining Gaussian distribution and homoscedasticity

Statistical data analysis

Wilcoxon's robust paired test was used for evaluation of the effect of finasteride treatment.

RESULTS

The sensitivity and specifity of the method was sufficient for the detection of dehydroepiandrosterone and its 7 (α and β)-hydroxy-derivatives as well in serum as in CSF from 3rd ventricle. Thus the concentrations of dehydroepiandrosterone, 7α-hydroxy-dehydroepiandrosterone, 7β-hydroxy-dehydroepiandrosterone, 5-androstene-3β,7α,17β-triol and 5-androstene-3β,7β,17β-triol were measured in cerebrospinal fluid from the third ventricle for the first time. The resulting concentrations for 15 patients are given in Table 4. The differencies of serum and CSF content of the steroids of postmenopausal women and men were insignificant. Table 5 shows the ratio of concentraions of free steroids in serum to steroids in CSF, conjugated steroids in serum to steroids in CSF and serum conjugates to serum free steroids. The levels of free dehydroepiandrosterone and 7-hydroxylated derivatives of dehydroepiandrosterone were somewhat higher in male ventricular fluid.

The ratio of serum to ventricular fluid concentations fluctuated in a very high range beeing lower for 7-oxygenated derivatives than for dehydroepiandrosterone, which might be due the contribution of 7-hyxdroxylation processes in brain tissue. Table 6 presents Pearson's correlation for DHEA and the 7α/β-hydroxy-steroids between CSF and serum unconjugated steroids, between CSF and serum steroid conjugates and between serum unconjugated steroids and serum steroid conjugates.

DISCUSSION

Endoscopic third ventriculostomy is a most effective treatment in cases of obstructive hydrocephalus that is caused by aqueductal stenosis and space-occupying

lesions. (Longatti *et al.* 2004, Feng *et al.* 2004; Hellwig *et al.* 2005). It also enables selective sampling of human ventricular CSF. However, until now only few reports on the hormone content in ventricular fluid were reported concerning mainly melatonin distribution in CSF (Longatti *et al.* 2004, 2007a,b, Tricoire *et al.* 2003). It could be shown on the case of melatonin that the concentration of this hormone in cerebrospinal fluid vary considerably according to the site of sampling. The third ventricle contains the highest concentration of melatonin and the choroid plexus and the pituitary recess the lowest (Longatti et al 2004, 2007a,b, Tricoire *et al.* 2003).

Naylor *et al.* (Naylor *et al.* 2008) demonstrated that dehydroepiandrosterone levels in cerebrospinal fluid are correlated with temporal cortex brain levels of this neurosteroids and that DHEA in cerebrospinal fluid may be relevant to the pathophysiology of Alzheimer´s disease. Another report on DHEA derivatives in CSF was published by Kim *et al.* (2003), who measured and compared CSF levels of DHEA, DHEAS, 7α-hydroxy-DHEA, 7β-hydroxy-DHEA, and 16α-hydroxy-DHEA in 14 patients with Alzheimer´s disease, 12 controls, and eight patients withvascular dementia. Results indicated that DHEAS CSF levels were significantly decreased in Alzheimerś disease and vascular dementia ($p < 0.007$), whereas other metabolite levels were not significantly changed. However, use of steroid level ratios resulted in significant differences between diseased and control patients. In addition, the 7α-hydroxy-DHEA/7β-hydroxy-DHEA ratio was significantly different between both types of dementia and could be used for differentiating Alzheimer´s diseae from vascular dementia.

Until now no attempt has been made for the determination of steroid hormones in ventricular CSF and this is the first report on the measurement of neuroactive steroid metabolites in ventricular CSF. When compared the concentration in serum and in ventricular CSF it seems that 7-oxygenated derivatives with the exception of 5-androstene-3β,7β,17β-triol penetrate in CSF more easily than free dehydroepinadrosterone itself. The cause may be either different transport over the blood-CSF barrier or an increased formation of 7-hydroxy-derivatives of dehydroepiandrosterone in the brain and secretion into ventricular CSF by choriodeal cells. However, the entry site of dehydroepiandrosterone metabolites into ventricular CSF and their role in this compartment are not known.

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