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THE EFFECT OF DEHYDROEPIANDROSTERONE SULFATE ADMINISTRATION ON THE 16α-HYDROXY-DEHYDROEPIANDROSTERONE EXCRETION IN CIRRHOTIC PATIENTS

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SUMMARY

Urinary 16α-hydroxy-dehydroepiandrosterone (16α-OH-DHA) and individual 11-deoxy-17-Ketosteroids (11-DO-17-KS) were determined by g.l.c. in 5 normal and 12 cirrhotic subjects before and after dehydroepiandrosterone sulfate (DHA-S) (50 mg) i.v. administration. Normal subjects increase the 11-DO-17-KS ($P < 0.02$) while cirrhotic patients increase the 16α-OH-DHA ($P < 0.005$) excretion after DHA-S, indicating that both groups metabolize the administered compound differently.

INTRODUCTION

Fotherby and coworkers [1] isolated 16α-hydroxy-dehydroepiandrosterone (16α-OH-DHA) from human urine for the first time in 1957. This compound seems to be a metabolite derived from dehydroepiandrosterone (DHA) or its sulphate (DHA-S) since hydroxylation in the 16α position may occur on both compounds [2]. In adults the amount of 16α-OH-DHA excreted in the urine is rather small [1, 3] while in newborns this compound predominates [3–6] reflecting probably the special characteristics of fetal metabolism.

The present work deals with the effects of DHA-S administration upon urinary 16α-OH-DHA and 11-deoxy-17-ketosteroids (11-DO-17-KS) excretion in normal and cirrhotic subjects.

The following abbreviations and trivial names have been used: Androsterone (A) = 3α-hydroxy-5α-androstan-17-one; Aetiocholanolone (Et) = 3α-hydroxy-5β-androstan-17-one; Dehydroepiandrosterone (DHA) = 3β-hydroxy-5-androsten-17-one; Dehydroepiandrosterone-sulfate (DHA-S) = 5-androsten-17-one-3β-yl-sulfate; 11-oxo-androsterone (O-A) = 3α-hydroxy-5α-androstane-11-17-dione; 11-oxo-aetiocholanolone (O-Et) = 3α-hydroxy-5β-androstane-11-17-dione; 11-hydroxy-androsterone (OH-A) = 3α, 11β-dihydroxy-5α-androstan-17-one; 11-hydroxy-aetiocholanolone (OH-Et) = 3α,11β-dihydroxy-5β-androstan-17-one; 16-hydroxy-dehydroepiandrosterone (16αOH-DHA) = 3α,16α-dihydroxy-5-androsten-17-one; Pregnanediol (P_2) = 5β-pregnane-3α,20α-diol; Pregnanetriol (P_3) = 5β-pregnane-3α, 17α, 20α-triol; Pregnanetriolone ($P_{3\text{-one}}$) = 3α,17α,20α-trihydroxy-5β-pregnan-11-one; Tetrahydro-11-deoxycortisol (THS) = 3α,17α,21-trihydroxy-5-pregnan-20-one; Tetrahydrocortisone (THE) = 3α, 17α, 21-trihydroxy-5β-pregnan-11, 20-dione; Tetrahydrocortisol (THF) = 3α, 11β, 17α, 21-tetrahydroxy-5β-pregnan-20-one; Cortolone-20α (C_{one}) = 3α, 17α, 20α, 21-tetrahydroxy-5β-pregnan-11-one; Cortol-20α (C_{ol}) = 5β-pregnane-3α, 11β, 17α, 20α, 21-pentol; Cholesterol-isobutyrate (S_t) = 5-cholesten-3β-yl-isobutyrate.

MATERIAL AND METHODS

The 24 h urine excretion of 5 normal and 12 cirrhotic subjects, aged 40–76 years, was collected before and after the 24 h i.v. administration of 50 mg DHA-S (Ro 6-6827/6, Lab. Hoffman-La Roche, Basel). Urine samples were stored at −20°C until analyzed.

The liver condition of all the patients had been confirmed by liver biopsy. They received no treatment during the week before the DHAS administration.

Steroid reference compounds were obtained from Ikapharm and Sigma, silylation reagents and enzymes for the hydrolysis from Serva, and the solvents and remaining reagents from Merck or Carlo Erba.

A part (10–40 ml) of the 24 h urine sample was hydrolyzed with β-glucuronidase-sulfatase for 24 h at 37°C. Following extraction of the freed steroids with 50 ml of ethylacetate–methylenechloride (1:1 v/v), the aqueous phase was acidified with hydrochloric acid to pH 1 and extracted continuously with 100 ml ethylether for 48 h. The extract was successively washed with 15 ml of disodium carbonate and water, pooled and dried down. Fifty μg of cholesterol isobutyrate were added to the residues to serve as the g.l.c. internal standard. Thereafter the extract was divided in two fractions for derivative formation.

The trimethylsilylderivatives (TMS) were formed with hexamethyldisilazane and trimethylchlorosilane at 65°C for 1 h as described by Gleispach *et al.* [7]. The methyloximes (MO) were formed with methoxylamine hydrochloride in pyridine overnight at room temperature [8]. After evaporation to dryness the TMS derivatives of the methyloximes (MO-TMS) are

Table 1. Relative retention time related to cholesterol-isobutyrate (t_r = 1) of several steroid compounds gas–liquid-chromatographed in OV-1 or OV-225 stationary phases either as trimetrylsilyl (TMS) or methoxyme-trimethylsilyl (MO-TMS) derivatives

Steroids	OV-1		OV-225	
	TMS	MO-TMS	TMS	MO-TMS
Androsterone (A)	0.113	0.129	0.161	0.112
Aetiocholanolone (Et)	0.117	0.131	0.188	0.125
Dehydroepiandrosterone (DHA)	0.137	0.155	0.224	0.152
Epiandrosterone	0.141	0.161	0.233	0.157
11-oxo-androsterone (O-A)	0.139	0.170	0.351	0.247
11-oxo-aetiocholanolone (O-Et)	0.139	0.170	0.397	0.271
11β-hydroxyandrosterone (OH-A)	0.176	0.203	0.450	0.301
11β-hydroxyaetiocholanolone (OH-Et)	0.176	0.203	0.524	0.337
16α-hydroxydehydroepiandrosterone (16α-OH-DHA)	0.223	0.227	0.243	0.152
Pregnanediol (P_2)	0.249	0.248	0.132	0.130
Pregnanetriol (P_3)	0.366	0.363	0.271	0.269
Pregnanetriolone ($P_{3\text{-one}}$)	0.470	0.466	0.594	0.597
3β-hydroxy-5β-pregnan-20-one	0.176	0.211	0.224	0.159
3β-hydroxy-5β-pregnan-20-one	0.182	0.220	0.252	0.179
5β-pregnano-3α, 11β, 17α, 20α-tetrol (P_4)	0.538	0.538	0.686	0.685
Tetrahydro-11-deoxycortisol (THS)	—	0.494	—	0.437
Tetrahydrocortisone (THE)	—	0.618	—	0.452
Tetrahydrocortisol (THF)	—	0.735	—	0.522
Cholesterol	0.581	0.581	0.329	0.330
Cortolone-20α (C_{one})	0.771	0.772	0.751	0.750
Cortol-20α (C_{ol})	0.892	0.894	0.891	0.893

formed as described previously. The MO-TMS once formed are partitioned between 1 ml of carbon disulphide and 0.6 ml of saturated NaCl solution, the aqueous phase being discarded and the organic phase evaporated to dryness and reconstituted with 0.1 ml of carbon disulphide. Four to five μl of each derivative solution are injected in the chromatograph. The g.l.c. analysis of each fraction was performed as previously described [9] in Fractovap (Carlo Erba) or Perkin–Elmer 990 instruments equipped with flame ionization detectors. The working conditions were as follows: the glass columns were 4 m long and 2 mm interior diameter with 3% OV-1 on Gas-Chrom Q for the MO-TMS analysis and 3% OV-225 also on Gas Chrom Q for the TMS derivatives. Carrier gas was nitrogen (40 ml/min) and the working temperature for the columns was 240°C. The detector responses to the different compounds in relation to the internal standard are the following: Androsterone-TMS = 1; Etiocholanolone-TMS = 1.1; DHA-TMS = 1.1 and 16α-OH-DHA-MO-TMS = 0.9.

RESULTS

The relative retention times of steroid TMS and MO-TMS derivatives are shown in Table I. The retention time of 16α-OH-DHA either as TMS or MO-TMS derivative does not coincide with that for any of the steroids listed in either of the gas–liquid chromatographic systems. Therefore its specific measurement should be possible with either derivative as shown in Fig. 1, where the principal steroid metabolites are simultaneously chromatographed.

The urinary excretion of Androsterone (A), Etiocholanolone (Et), DHA and 16α-OH-DHA both in normal and cirrhotic patients before and after DHA-S administration are shown in Table 2. The 11-deoxy-17-ketosteroids (11-DO-17-KS) fraction represents the sum of A, Et and DHA values. In basal condition there is no difference in urinary excretion between normal and cirrhotic subjects except for Et excretion ($P < 0.05$). The increase of A after DHA-S administration become statistically different for both groups ($P < 0.05$). The administration of 50 mg DHA-S in control subjects induces an increment of 11-DO-17-KS ($P < 0.1$) while the 16α-OH-DHA excretion is not modified ($P < 0.975$).

In cirrhotic patients, on the contrary the increase of 11-DO-17-KS is less marked ($P < 0.2$) while the 16α-OH-DHA excretion increases significantly ($P < 0.001$). Comparing the post-DHA-S urinary excretion patterns in normal and cirrhotic subjects it becomes clear that the latter excrete less 11-DO-17-KS ($P < 0.02$) and more 16α-OH-DHA ($P < 0.005$) than the former ones.

DISCUSSION

The g.l.c. behaviour of the TMS and MO-TMS derivatives of 16α-OH-DHA in two stationary phases of different polarity (Table 1) supports the assumption that the material measured under the peak having the retention time of the reference 16α-OH-DHA is really this steroid.

The quantitation of the 16α-OH-DHA can reasonably be carried out as either derivative using OV-1

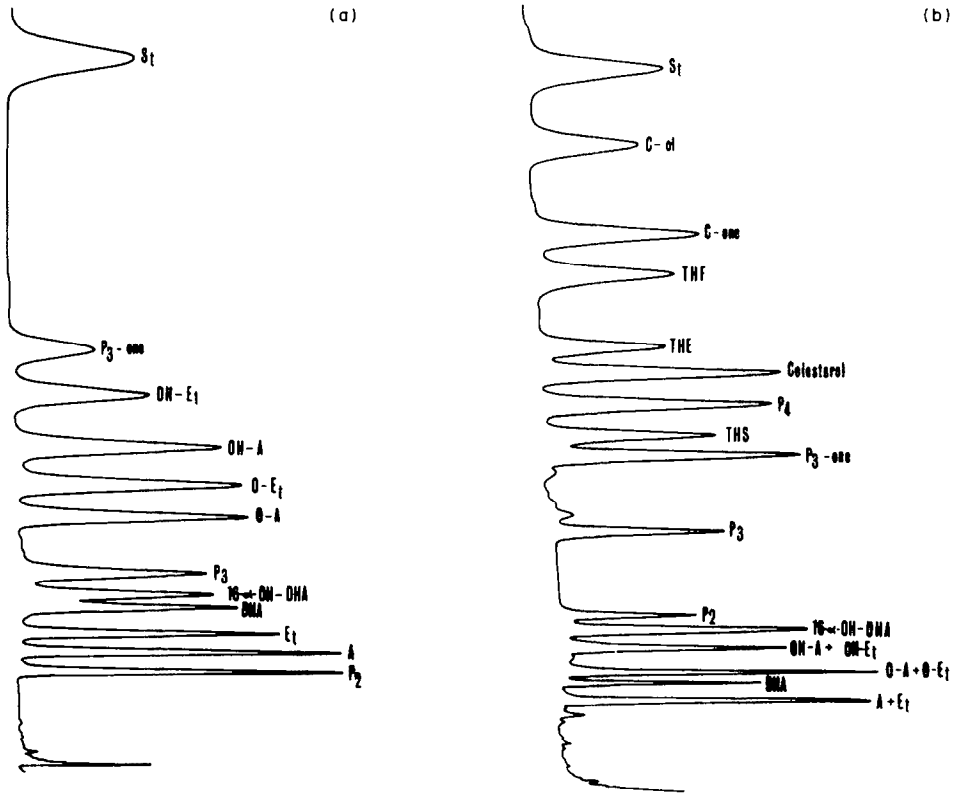

Fig. 1. Gas–Liquid chromatograms obtained with reference compounds as TMS derivatives in OV-225 (a) and MO-TMS derivatives in OV-1 (b). For the abbreviations see footnote on page 1.

Table 2. Urinary excretion values expressed as ug/24 h of the compounds: androsterone (A), aetiocholanolone (Et), dehydroepiandrosterone (DHA), 16α-hydroxydehydroepiandrosterone (16α-OH-DHA) and the sum of A + Et + DHA (11-DO-17-KS) before and after the administration of 50 mg DHA-S i.v. in normal and cirrhotic subjects. $\bar{x}$ = mean value; (s.e.m.) = standard error of the mean

	Basal					Post-DHA-S				
	A	Et	DHA	11-dO 17-KS	16α-OH DHA	A	Et	DHA	11-dO 17-KS	16α-OH DHA
Normal										
1	1737	654	348	2739	110	1850	709	2727	5286	94
2	787	1221	<20	2018	<20	1593	1692	33	3318	<20
3	70	188	<20	268	20	783	1099	459	2341	37
4	724	581	<20	1315	169	738	236	472	1446	150
5	775	610	<20	1395	<20	1549	1579	61	3189	<20
$\bar{x}$	818.6	650.8	77.6	1547.0	61.8	1302.6	1063.0	750.4	3116.0	60.2
sem	266.0	165.2	67.5	409.6	33.0	227.3	271.3	502.9	638.1	27.1
Cirrhotic										
1	<20	<20	<20	30	70	<20	<20	<20	30	851
2	<20	<20	<20	30	18	612	<20	<20	632	176
3	37	134	<20	181	<20	366	269	421	1056	150
4	179	98	<20	287	80	328	236	19	583	444
5	288	105	<20	403	24	387	53	<20	450	526
6	637	149	<20	796	<20	1187	618	35	1840	<20
7	2066	264	<20	2340	<20	2102	916	370	3388	291
8	122	81	<20	213	<20	316	114	36	486	190
9	403	223	23	649	<20	1099	291	39	1429	555
10	208	433	<20	651	<20	115	250	20	375	<20
11	224	1296	<20	1530	<20	580	2960	20	3550	736
12	288	184	<20	482	<20	686	476	39	1201	262
$\bar{x}$	372.6	248.9	11.0	632.6	22.6	649.0	516.9	85.7	1251.6	350.0
sem	162.3	100.9	1.0	195.5	7.1	166.6	235.0	42.1	332.4	78.8

or OV-225 stationary phases. Nevertheless in the OV-1 system the separation is better and we usually utilize the MO-TMS derivatives in such columns since it allows at the same time to measure the tetrahydro metabolites of the corticosteroids present in the sample [9].

Usually, the changes induced by the DHA-S administration are obscured by the large variations in A, Et and DHA excretion to such an extent that only when taken as a group (11-DO-17-KS) become statistically significant ($P < 0.05$).

It seems remarkable that only around 5% of the administered DHA-S mass appears in the urine during the first 24 h in form of the detected metabolites. This could be due in part to an insufficient hydrolysis of the urinary conjugates. Nevertheless, Baulieu and coworkers [10] found that only 25% of the radioactivity injected as DHA-S-H^3 appeared in the urine over a four day period. This finding contrasted with the quantitative recovery observed by McDonald *et al.* [11] (93%) after DHA administration. This difference pointed out the different metabolic fate of the free and sulfoconjugated DHA compounds in spite of their reversible interconversion [12]. The biliary excretion of DHA-S has been shown to be 4–6 times higher than for free DHA [13] indicating the importance of the liver in the clearance and excretion of the sulfoconjugated compound.

In normal men the exogenous DHA-S induces no changes in the 16α-OH-DHA excretion. There may be two explanations for it: either the existing alternative metabolic pathways, being capable of handling the exogenous DHA-S offered, do not allow an increase of the substrate to the 16α-hydroxylase, or this enzyme being normally saturated cannot increase the turnover of the increased substrate offered.

No differences can be established between the basal urinary steroid patterns of normal and cirrhotic patients probably because individual variability obscures them. Nevertheless in cirrhotic subjects DHA-S administration is not reflected as an increase of 11-DO-17-KS fractions.. This metabolic limitation could be due to multiple alternative possibilities such as: (a) decreased sulfatase activity and facilitation of the biliary over the urinary excretion of the DHA-S; (b) increase conversion of DHA to Androstenedione with decreased ring A reduction or (c) decreased ring B reduction of either free DHA or its conjugates excreted in the urine. Other alternative causes of the reported findings could be: prolonged half life of DHA-S, changes in its distribution and/or fecal excretion of DHA-S or the existence of other unknown metabolic pathways of DHA-S.

Nevertheless, the increased 16α-OH-DHA excretion in cirrhotic patients after DHA-S administration can be due to either an increased 16α-hydroxylase activity or to the increased availability of substrate for the unchanged enzyme. The second possibility is in keeping with the fact that DHA-S constitutes a better substrate for 16α-hydroxylase than the free steroid [14] and the possibility of a decreased sulfatase activity in the damaged liver of the cirrhotic patients, resembling the functional situation of the fetal liver [15]. It should be pointed out that, Zumoff *et al.* [16] studied the estradiol metabolism in cirrhotic patients and found a 65% increase of the 16α-hydroxylated compounds appearing in their urine with a decreased excretion of the other metabolites.

If the excretion of 11-DO-17-KS after DHA-S administration represents a decreased hepatic capacity to metabolize this compound it may well be that more substrate may be available for the 16α-hydroxylase to act upon. The question then arises concerning the localization of this enzyme. If it is primarily in the liver cells, it seems peculiar that in cirrhotic patients the main steroid metabolizing enzymes should be affected while the 16α-hydroxylase remains intact, since there is no known biological role for this enzyme in the adult being.

There is also the possibility of an extrahepatic localization of 16α-hydroxylase which only manifests itself in cases of diminished liver function. Such an extrahepatic localization has been described for the fetal adrenal gland and the adult gland may also retain certain activity. Other tissues like the human breast tumors have been shown to contain also 16α-hydroxylase [17].

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