Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 903–905. Pergamon Press. Printed in Great Britain

INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY STEROIDS

VI – EFFECTS OF NEW SYNTHETIC DEHYDROEPIANDROSTERONE CONJUGATES UPON THE ACTIVITY OF HUMAN PLACENTAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE

G. W. OERTEL and P. BENES
Abteilung für Experimentelle Endokrinologie, Universitäts-Frauenklinik, D-65 Mainz, Germany

(*Received* 27 *April* 1972)

SUMMARY

New synthetic DHEA conjugates were tested for their capacity as inhibitors of purified human placental G-6-PDH. Although most of these derivatives like DHEA alkyl sulfates, alkyl sulfonates and alkyl phosphates were found to be more effective than free DHEA, their inhibitory activity did not equal that of DHEA sulfatide. The relationship between the structure of such conjugates and their biological activity is discussed.

ONLY recently the effects of dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one, DHEA), its natural conjugates and several derivatives upon human placental glucose-6-phosphate dehydrogenase (G-6-PDH) have been reported [1]. These experiments indicated that such anionic conjugates as DHEA sulfate, phosphate or glucuronoside do not inhibit G-6-PDH. On the other hand, DHEA sulfatide, which is assumed to represent the endogenous sulfoconjugate of DHEA [2, 3], proved to be the most potent inhibitor of this enzyme by far [1, 4]. Since other lipophile derivatives of DHEA like DHEA laurate or stearate exhibited practically no activity in the enzyme inhibition test, the inhibitory activity does not depend on lipophile properties of the steroid or steroid conjugate but may be attributed rather to specific structural elements of the molecule. The esterification of DHEA with sulfatidic acid (diglyceride sulfuric acid), yielding the steroid sulfatide, apparently provides the optimal structure for the allosteric inhibition of G-6-PDH.

In order to gain further information on the relationship between structure and physiological activity of DHEA conjugates, new synthetic compounds tested for their inhibitory effects upon human placental G-6-PDH.

MATERIALS AND METHODS

Purified human placental G-6-PDH was obtained by procedures outlined in a preceding publication [5].

The synthesis of new DHEA derivatives, which will be described in a forthcoming communication, was performed as follows:

(a) DHEA alkyl sulfates were prepared from DHEA and alkyl sulfuric acid by the carbodiimide method in dimethylformamide/acetone, by condensation of alkyl iodide with the silver salt of DHEA sulfate in acetone, and from DHEA sulfuric acid and the appropriate alkanol by the carbodiimide method.

(b) DHEA hexadecane sulfonate was synthesized from DHEA and hexadecane sulfonyl chloride in dry pyridine.

(c) DHEA alkyl phosphates were prepared from DHEA phosphoric acid and

the corresponding alkanol by the carbodiimide method in dimethylformamide/acetone.

All DHEA derivatives were purified by recrystallization and/or preparative thin-layer chromatography on silica gel in cyclohexane, on cellulose in methanol–ether (18 : 2 v/v) or on polyamide in hexane. The composition of the various compounds was verified by elemental analysis as well as by quantitative estimation of DHEA [6] and sulfate [7] or phosphate [8] (Table 1).

Enzyme inhibition tests were performed with 0·1 ml of the purified enzyme preparation, to which 3·0 ml 0·05 M triethanolamine/0·005 M EDTA buffer of pH 7·6, 0·1 ml 0·01 M NADP solution and 0·02 ml dioxane with steroid derivative were added. The final concentration of the derivatives corresponded to a 10^{-5} M or 10^{-6} M solution. After addition of varying amounts of glucose-6-phosphate as substrate in 0·05 ml water changes in the absorbance at 366 nm were registered over 10 min at 25°C and compared to those of the corresponding blank, e.g. without steroid derivative. All results were evaluated by the method of Hunter and Downs [9].

Table 1. Synthetic DHEA conjugates used in the present investigation

Compound	m.p.†	calculated for: formula	calculated for: % S (P)	Analysis found % S (P)	uMol DHEA/ uMol $SO_4(PO_4)$
DHEA hexyl sulfate	112° d	$C_{25}H_{40}O_5S$	7·09	6·92	1·00/1·06
dodecyl sulfate	114° d	$C_{31}H_{52}O_5S$	5·98	5·74	1·00/1·04
octadecyl sulfate	115° d	$C_{37}H_{64}O_5S$	5·17	5·02	1·00/1·05
octadecanoyl glycol sulfate	57° d	$C_{39}H_{66}O_7S$	4·73	4·46	1·00/0·97
O-dihexadecyl glycero sulfate	96° d	$C_{54}H_{98}O_7S$	3·59	3·33	1·00/0·96
hexadecane sulfonate	74°	$C_{35}H_{60}O_4S$	5·56	5·77	
dioctadecanoyl glycero phosphate	140°	$C_{58}H_{103}O_9P$	3·30	3·19	1·00/0·94
O-dihexadecyl glycero phosphate	106°	$C_{54}H_{99}O_7P$	3·45	3·29	1·00/0·95

†Uncorrected.
d Decomposition.

RESULTS

The inhibition of human placental G-6-PDH by synthetic conjugates of DHEA at 10^{-5} M or 10^{-6} M inhibitor concentrations is shown in Table 2. This Table 2 also contains the K_i-values of the various derivatives for glucose-6-phosphate as substrate.

DISCUSSION

As can be derived from Table 2 all synthetic DHEA conjugates except DHEA hexyl sulfate turned out to be more effective inhibitors of human placental G-6-PDH than the free steroid. At the same time these findings also exclude the presence of mere mixtures of lipid material and DHEA, DHEA sulfate or phosphate and support the identity of the synthetic compounds. The admixture of lipids to free DHEA, its sulfate or phosphate does not influence the effects of

Table 2. Inhibition of human placental G-6-PDH by synthetic DHEA conjugates

Compound	Inhibition at: 10^{-5} M	10^{-6} M	K_i-value for G-6-P
DHEA hexyl sulfate	29	13	$0{\cdot}92 \times 10^{-5}$ M
dodecyl sulfate	55	20	0·74
octadecyl sulfate	64	24	0·63
octadecanoyl glykol sulfate	66	22	0·57
O-dihexadecyl glycero sulfate	68		0·55
hexadecane sulfonate	54	17	0·78
dioctadecanoyl glycero phosphate	71	26	0·41
O-dihexadecyl glycero phosphate	61		0·68
DHEA	53	14	0·79
DHEA sulfate	7	3	31
DHEA dihexadecanoyl glycero sulfate	82	30	0·36

these substances upon G-6-PDH [1]. The activity of DHEA sulfatide in the G-6-PDH inhibition test, however, obviously exceeds that of the synthetic new derivatives. Concerning a structure-activity relationship the formation of a covalent ester bond between DHEA sulfuric or phosphoric acid and aliphatic alcohols obviously converts the inactive DHEA sulfate or phosphate into most active inhibitors of G-6-PDH. Since in free DHEA the equatorial hydroxy group at C-3 of the planar steroid molecule appears to be vital for its inhibitory activity, it may be assumed that the sulfuric or phosphoric acid diester structure confers a certain negative charge to this oxygen. Such a structure probably facilitates the interaction of steroid conjugate and enzyme, resulting in the conformational changes of the protein molecule and the partial inactivation of the enzyme. Regarding the different alkyl sulfates of DHEA the increase in the chain length of the esterified alkanols from C_6 to C_{12} and C_{18} promoted the activity together with the lipophile character. Conversely, the equally lipophile DHEA stearate exhibited less activity in the enzyme inhihition test than DHEA caproate or even DHEA acetate [1]. Here, the aliphatic chain seems to prevent the participation of the oxygen at C-3 in the attachment of the steroid derivative to the enzyme molecule. Distinct differences in the biological activity were also observed upon modification of the alkanol moiety from monohydroxy to substituted dihydroxy and trihydroxy alcohols.

On the basis of their pronounced activity in the G-6-PDH inhibition test such compounds as DHEA octadecyl sulfate, octadecanoyl glycol sulfate or dioctadecanoyl glycero phosphate may be considered for therapeutic application as depressors of this enzyme in cases with G-6-PDH hyperactivity [10].

REFERENCES

1. Benes P. and Oertel G. W.: *J. steroid Biochem.* **3** (1972) 799.
2. Oertel G. W.: *Biochem. Z.* **334** (1961) 431.
3. Oertel G. W.: *Hoppe–Seyler's Z. physi l. Chem.* **343** (1966) 276.
4. Oertel G. W. and Menzel P.: *Exc. Med. Int. Congr. Ser.* **210** (1970) 163.
5. Menzel P.. Gobbert M. and Oertel G. W.: *Hormone Metab. Res.* **2** (1970) 225.
6. Treiber L. and Oertel G. W.: *Z. klin. Chem. klin. Biochem.* **5** (1967) 83.
7. Minami E. and Watanuki K.: *Japan. Analyst* **8** (1959) 175.
8. Chen P. S., Topibara T. Y. and Warner H.: *Anal. Chem.* **11** (1956) 1756.
9. Hunter A. and Downs C. E.: *J. biol. Chem.* **157** (1945) 427.
10. Sonka J., Gregorova I., Jiranek M., Kölbel F. and Matys Z.: *Endokrinologie* **47** (1965) 152.