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INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY STEROIDS

IV – EFFECTS OF C_{18}-STEROIDS UPON HUMAN PLACENTAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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SUMMARY

By incubation of purified human placental glucose-6-phosphate dehydrogenase with C_{18}-steroids it could be demonstrated that only 17-oxo compounds such as estrone, 2-methoxy-estrone, 16α-hydroxy-estrone and 16-keto-estrone caused a significant inhibition of the enzyme. Concerning a relationship between inhibitory activity and chemical structure of estrogens the present results confirm previous findings, obtained with C_{19}-steroids.

INTRODUCTION

As SHOWN by earlier experiments[1–3] the inhibition of human glucose-6-phosphate dehydrogenase (G-6-PDH) by steroids depends on the structure of the steroid molecule. Maximal enzyme inhibition was obtained with planar C_{19}- and C_{21}-steroids (5α-, 4-ene- or 5-ene-steroids), containing a 17- or 20-oxo group and an equatorial hydroxy group at C-3. Additional functional groups in such molecules greatly reduced the inhibitory properties.

Since also estrogens exhibit a planar configuration by virtue of their aromatic ring A the effect of various phenolic steroids upon the activity of human placental G-6-PDH was investigated.

EXPERIMENTAL

Purified human placental G-6-PDH was prepared as outlined in a preceding communication[4]. The following C_{18}-steroids, purchased from IKAPHARM, Ramat-Gan, Israel, were assayed for their inhibitory activity:

estrone	3-hydroxy-1,3,5(10)-estratrien-17-one
2-methoxy-estrone	3-hydroxy-2-methoxy-1,3,5(10)-estratrien-17-one
16α-hydroxy-estrone	3,16α-dihydroxy-1,3,5(10)-estratrien-17-one
16-keto-estrone	3-hydroxy-1,3,5(10)-estratriene-16,17-dione
3-hydroxy-estratetraene	1,3,5(10),16-estratetraen-3-ol
estradiol-17β	1,3,5(10)-estratriene-3,17β-diol
estradiol-17α	1,3,5(10)-estratriene-3,17α-diol
2-methoxy-estradiol	2-methoxy-1,3,5(10)-estratriene-3,17β-diol
6α-hydroxy-estradiol	1,3,5(10)-estratriene-3,6α,17β-triol
estriol	1,3,5(10)-estratriene-3,16α,17β-triol
16-epi-estriol	1,3,5(10)-estratriene-3,16β,17β-triol
17-epi-estriol	1,3,5(10)-estratriene-3,16α,17α-triol
16,17-epi-estriol	1,3,5(10)-estratriene-3,16β,17α-triol

2-methoxy-estriol — 2-methoxy-1,3,5(10)-estratriene-3,16α,17β-triol
6-hydroxy-equilenin 3,6-dimethylether — 3,6-dimethoxy-1,3,5(10),6,8-estrapentaen-17-one.

The inhibition tests were performed with 0·1 ml of the enzyme preparation, 2·9 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 dioxan, containing the steroid. The final concentration of estrogens corresponded to a 10^{-5} M solution. After additon of varying concentrations of G-6-P in 0·05 ml water changes in the absorbance at 366 nm were registered over 10 min at 25°C and compared to those observed with a corresponding blank, that is without steroid. For estimation of K_i-values all results were evaluated by the method of Hunter and Downs [5].

RESULTS AND DISCUSSION

The results of the enzyme inhibition tests are compiled in Table 1, presenting the K_i-values of the various estrogens for G-6-P as substrate. From these figures it becomes apparent that only estrone, 2-methoxy-estrone, 16α-hydroxy-estrone and 16-keto-estrone produced a significant inhibition of the placental enzyme. Whereas at a 10^{-5} *M* concentration dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one) caused a 53% inhibition of placental G-6-PDH [3] the most

Table 1. Inhibition of human placental G-6-PDH by C_{18}-steroids

Steroid	K_i-value for G-6-P
estrone	$2{\cdot}7 \times 10^{-5}$ *M*
16α-hydroxy-estrone	3·1
16-keto-estrone	4·0
2-methoxy-estrone	6·0
3-hydroxy-estratetraene	29
estradiol-17β	46
estradiol-17α	49
6α-hydroxy-estradiol	54
estriol	57
16-epi-estriol	58
17-epi-estriol	58
16,17-epi-estriol	73
2-methoxy-estradiol	87
2-methoxy-estriol	88
6-hydroxy-equilenine 3,6-dimethyl ether	89

active estrogen, e.g. estrone merely led to a 32% inhibition. By reduction of the 17-oxo group in estrone to the 17β- or 17α-hydroxy group the inhibitory activity is almost completely lost. Such findings confirm previous data, gained with 17-oxo and 17-hydroxy C_{19}-steroids [1, 2]. Additional functional groups in ring D (16α-hydroxy-estrone) reduced the inhibitory activity to a lesser degree than additional substituents in ring A (2-methoxy-estrone), stressing the importance of structural elements at this end of the molecule. As it appears a certain

negative charge of the oxygen at C-3 is required for attachment of the steroid to the protein molecule.

Since the more active estrogens like estrone do not even reach a 10^{-7} *M* concentration in peripheral human plasma[6] it seems quite unlikely that estrogens participate in the regulation of G-6-PDH under physiological conditions.

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