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INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY STEROIDS

II. EFFECTS OF 17-OXO C_{19}-STEROIDS UPON HUMAN PLACENTAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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SUMMARY

The assay of human placental glucose-6-phosphate dehydrogenase in the presence of various 17-oxo C_{19}-steroids confirmed that the oxo group at C-17 as well as an oxygen function at C-3 are required for significant inhibition of the enzyme. The spacial arrangement of the oxygen function at C-3 in relation to the general plane of the steroid molecule appears to be of major importance for the inhibitory properties of 17-oxo steroids. Maximal inhibition was obtained with 3β-hydroxy-5α-androstan-17-one. Additional hydroxy or oxo groups in ring C decreased the inhibitory activity of the particular C_{19}-steroids.

INTRODUCTION

In a previous communication [1] the effect of 3β-substituted, ring A or B unsaturated C_{19}- and C_{21}-steroids upon the activity of human red blood cell glucose-6-phosphate dehydrogenase (G-6-PDH) has been described. From these investigations it could be concluded that in addition to the oxo group at C-17 or C-20 in the various Δ^4- or Δ^5-steroids a 3-hydroxy group will provide optimal activity of the steroid in the enzyme inhibition test.

In continuation of these experiments other 17-oxo C_{19}-steroids were tested as potential inhibitors of human placental G-6-PDH.

EXPERIMENTAL

The placental G-6-PDH was prepared essentially as outlined in a recent publication [2]. Purification was achieved by an ammonium sulfate precipitation at 30% and 50% saturation, followed by the adsorption of the latter precipitate on calcium phosphate gel and another ammonium sulfate precipitation with the gel eluate at 35% and 55% saturation. The final precipitate was dissolved in 0·05 *M* triethanolamine/0·005 *M* EDTA buffer at pH 7·6 and submitted to the standard enzyme test [1]. The latter was performed in duplicate, using 2.95 ml of the above buffer, 0·1 ml of the enzyme preparation, 0·1 ml 0·03 *M* NADP and varying concentrations of glucose-6-phosphate (G-6-P) in 0·05 ml solution. The 17-oxo C_{19}-steroids listed in Table 1* were added in 0·02 ml dioxan. All other experimental details as well as the procedure selected for evaluation of the results were the same as those given in the first communication [1].

RESULTS

The supernatant, obtained by centrifugation of the placental homogenate at 15,000 **g**, exhibited a specific activity of 12·8 mU/mg protein. After purification the specific activity increased to 1940 mU/mg protein, indicating a 152-fold enrichment of the enzyme. The K_M-value of the purified enzyme amounted to $9{\cdot}2 \times 10^{-5}$ *M* with G-6-P as substrate.

*The steroids used were obtained from Mann Research Laboratories, New York, N.Y., U.S.A. and from Ikapharm, Ramat-Gan, Israel.

The inhibition of the purified enzyme by 10^{-5} M and 10^{-6} M concentrations of the different 17-oxo steroids is shown in Fig. 1. The K_i-values of the individual compounds, derived from the Hunter–Downs plot[3] of varying concentrations of G-6-P vs. $i(\alpha/(1-\alpha))$ (i = inhibitor concentration, $\alpha = \Delta E$ in the presence of inhibitor/ΔE in the absence of inhibitor) are listed in Table 1.

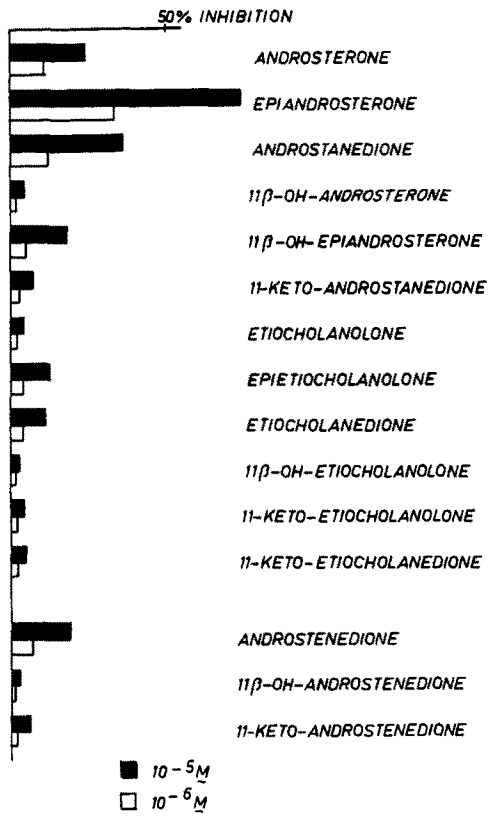

Fig. 1. Inhibition of human placental glucose-6-phosphate dehydrogenase by 17-oxo C_{19}-steroids.

DISCUSSION

The present data obviously support the conclusions drawn from the earlier experiments with 3β-substituted Δ^5-steroids. 3β-Hydroxy-5α-androstan-17-one proved to be an even more efficient inhibitor of human placental G-6-PDH than 3β-hydroxy-5-androsten-17, the K_i-value of which amounted to $6{\cdot}9\times10^{-6}$ M.* The lack of activity seen with 5-androstane-3β,17β-diol, in contrast to the findings of Betz and Warren[4], only stresses the importance of the 17-oxo group. In that respect it may be speculated that in the less purified enzyme preparation of these authors a partial oxidation of 5α-androstane-3(?),17β-diol to the corresponding 17-oxo steroid could have taken place, mediated through contaminating 17β-hydroxysteroid dehydrogenase and eventually leading to significant inhibition of

*Since the K_i-value of the latter steroid resembled that obtained with RBC G-6-PDH ($6{\cdot}2\times10^{-6}$ M)[1], the origin of the enzyme seems to be of little importance for the evaluation of both investigations.

Table 1. Inhibition of human placental G-6-PDH by 17-oxo C_{19}-steroids

Steroid	Abbreviations used in Fig. 1	K_i-value for G-6-P
3β-hydroxy-5α-androstan-17-one	Epiandrosterone	$0{\cdot}28 \times 10^{-5}$ M
5α-androstane-3,17-dione	Androstanedione	1·9
3α-hydroxy-5α-androstan-17-one	Androsterone	4·2
3β,11β-dihydroxy-5α-androstan-17-one	11β-OH-epiandrosterone	4·6
3β-hydroxy-5β-androstan-17-one	Epietiocholanolone	8·7
5β-androstane-3,17-dione	Etiocholanedione	9·3
3α-hydroxy-5α-androstane-11,17-dione	11-keto-androsterone	13
5β-androstane-3,11,17-trione	11-keto-etiocholanedione	21
3α,11β-dihydroxy-5α-androstan-17-one	11β-OH-androsterone	24
3α-hydroxy-5β-androstan-17-one	Etiocholanolone	36
3α-hydroxy-5β-androstane-11,17-dione	11-keto-etiocholanolone	38
3α,11β-dihydroxy-5β-androstan-17-one	11β-OH-etiocholanolone	88
4-androstene-3,17-dione	Androstenedione	3·0
4-androstene-3,11,17-trione	11-keto-androstenedione	23
11β-hydroxy-4-androstene-3,17-dione	11β-OH-androstenedione	56

the enzyme by the reaction product. The oxidation of the equatorial 3β-hydroxy group in 3β-hydroxy-5α-androstan-17-one to the 3-oxo group resulted in a marked decrease of activity. This loss of activity is still more pronounced in 3α-hydroxy-5α-androstan-17-one. On the other hand, in the 5β-androstane series the 3β-hydroxy-17-oxo compound (3β-hydroxy-5β-androstan-17-one) was found to be more active in the enzyme inhibition test than its epimer 3α-hydroxy-5β-androstan-17-one. It therefore appears that the spatial arrangement of the oxygen function is more vital for inhibitory properties than its nature. All results are consistent with the hypothesis that an oxo group at C-17 (or C-20) and a 3-oxygen function within the general plane of the steroid molecule are optimal features for its inhibitory properties. The introduction of a double bond at C-4 [5], which reduces the chair conformation of ring A to a half-chair conformation with a rather planar arrangement of C-3, C-4, C-5 and C-6 (C-7) obviously places 4-androstene-3,17-dione between 5α-androstane-3,17-dione and 5β-androstane-3,17-dione. As a matter of fact, the K_i-values of these three steroids are in agreement with such a hypothesis. The influence of additional hydroxy groups or oxo groups in ring C is demonstrated by higher K_i-values of 11β-hydroxy or 11-oxo derivatives of the parent compounds. This effect of additional substituents, however, can be considered minor as compared to that of similar substituents at C-7 or C-19 in 3β-hydroxy-Δ^5-steroids. The loss of activity caused by an additional hydroxy group always exceeded that produced by the corresponding oxo group.

Taking into account all available data on the activity of C_{19}- and C_{21}-steroids as inhibitors of human G-6-PDH it may be summarized that numerous compounds are potential inhibitors of this enzyme in the *in vitro* test. Under physiological conditions, conversely, only a very few of these occur in high enough quantities as to be able to participate in the regulation of G-6-PDH.

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