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INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY STEROIDS

I. EFFECTS OF 3β-HYDROXY-Δ⁵-STEROIDS OF THE C_{19}- AND C_{21}-SERIES UPON HUMAN RED BLOOD CELL GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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SUMMARY

Various 3β-substituted Δ⁵-(Δ⁴) C_{19}- or C_{21}-steroids were tested as inhibitors of human red blood cell glucose-6-phosphate dehydrogenase. From the results obtained it was concluded that the presence of several structural features is required for optimal activity: an oxo group at C-17 or C-20 and a 3-hydroxy group. Additional hydroxy or oxo groups near these centers caused a more or less pronounced decrease in inhibitory activity.

INTRODUCTION

IT SEEMS well established that the activity of human red blood cell (RBC) glucose-6-phosphate dehydrogenase (G-6-PDH) is inhibited by various steroids [1–3]. According to Marks and Banks [1] this inhibition depends mainly on the presence of an oxo group at C-17 or C-20 of the steroid molecule, whereas additional hydroxy or oxo groups at C-3 and double bonds in ring A or B were considered to be of little effect. On the other hand, experiments with human placental G-6-PDH [4] suggested that hydroxy groups at C-3 are involved in the inhibition of this enzyme, the 3β-hydroxy-Δ⁵-steroids being the most potent inhibitors.

In order to obtain further information on a possible structure-activity relationship a series of unsaturated, 3β-substituted C_{19}- and C_{21}-steroids were tested as inhibitors of RBC G-6-PDH.

EXPERIMENTAL

Human RBC G-6-PDH was purified essentially as described by Marks *et al.* [2]. The enzyme tests were performed by routine procedures [5], using 3·00 ml of 0·05 *M* triethanolamine/0·005 *M* EDTA buffer of pH 7·6, 0·05 ml of the purified enzyme in said buffer, 0·10 ml 0·03 *M* NADP and varying concentrations of glucose-6-phosphate (G-6-P) in 0·05 ml solution. After 5 min preincubation of the enzyme at 25°C in the presence of NADP and of each of the steroids listed in Table 1*, added in 0·02 ml dioxan, the substrate G-6-P was introduced and the changes in extinction at 366 nm recorded every minute. The velocity of $NADPH_2$ formation was compared with that observed in the absence of steroid and the inhibition of the enzyme calculated in per cent. For determination of K_I-values, the results received with decreasing concentrations of G-6-P in the presence of constant amounts of steroid and enzyme and an excess of NADP were evaluated by the methods of Hunter and Downs [6].

*The steroids used in this investigation were obtained from Mann Research Laboratories, New York, N.Y., U.S.A., from Ikapharm, Ramat-Gan, Israel or through the courtesy of Dr. M. Gut, Worcester Foundation for Experimental Biology, Shrewsbury, Mass., U.S.A.

Table 1. Inhibition of human RBC G-6-PDH by 3β-substituted ring A or B unsaturated C_{19}- and C_{21}-steroids

Steroid	Abbreviations used in Figs. 1 and 2	K_i-value for G-6-P
3β-hydroxy-5-androsten-17-one	DHEA	$0{\cdot}62 \times 10^{-5}$ M
3β,16α-dihydroxy-5-androsten-17-one	16α-OH-DHEA	0·66
3β-hydroxy-4-androsten-17-one	3β-OH-4-androsten-17-one	0·86
3β-hydroxy-5-androstene-16,17-dione	16-keto-DHEA	5·2
3β,7β-dihydroxy-5-androsten-17-one	7β-OH-DHEA	5·9
3β,7α-dihydroxy-5-androsten-17-one	7α-OH-DHEA	8·2
3β,19-dihydroxy-5-androsten-17-one	19-OH-DHEA	12
3β-chloro-5-androsten-17-one	3β-chloro-DHEA	14
3β-hydroxy-5-androstene-7,17-dione	7-keto-DHEA	18
3β,17β-dihydroxy-5-androsten-16-one	16-keto-androstenediol	29
5-androstene-3β,17β-diol	Androstenediol	45
5-androstene-3β,16α,17β-triol	Androstenetriol	58
3β-hydroxy-5-pregnen-20-one	Pregnenolone	0·71
3β,21-dihydroxy-5-pregnen-20-one	21-OH-pregnenolone	0·78
3β,17α-dihydroxy-5-pregnen-20-one	17α-OH-pregnenolone	1·2
3β,17α,21-trihydroxy-5-pregnen-20-one	17α,21-OH-pregnenolone	2·3
5-pregnene-3β,20α-diol	Pregnenediol-20α	20
5-pregnene-3β,20β-diol	Pregnenediol-20β	48
5-pregnene-3β,17α,20α-triol	Pregnenetriol	76

RESULTS

Before purification of the enzyme the specific activity was found to be 0·188 mU/mg protein. The purified RBC G-6-PDH exhibited a specific activity of 72·6 U/mg protein, indicating a 386-fold purification. The K_M-value of the purified enzyme turned out to be $6{\cdot}9 \times 10^{-5}$ M with G-6-P as substrate. The inhibition of purified G-6-PDH by different concentrations of the various steroids is shown in Figs. 1 and 2. Table 1 contains the corresponding K_i-values, which were obtained with 10^{-5} M concentrations of the individual compounds.

DISCUSSION

All results, presented in Figs. 1 and 2 as well as in Table 1, are definitely not consistent with the hypothesis that only one common structural feature like an oxo group at C-17 or C-20, or a 3β-hydroxy-Δ^5-grouping is responsible for the inhibitory activity of the steroid. Instead, the evaluation of the foregoing experimental data reflects multiple structural requirements for optimal activity.

Since 5-androstene-3β,17β-diol, 5-androstene-3β,16α,17β-triol and 3β,17β-dihydroxy-5-androsten-16-one, as well as analogous C_{21}-steroids such as 5-pregnene-3β,20α-diol and 5-pregnene-3β,17α,20α-triol proved to be without significant effect upon human RBC G-6-PDH, it may be safely assumed that the oxo group at C-17 or C-20 is necessary for the inhibition of the enzyme. In contrast to the findings of Betz and Warren[4], 5α-androstane-3α,17β-diol also did not exhibit inhibitory properties, thus confirming such a conclusion. The importance of substituents at C-3 is borne out by the fact that a 3-hydroxy group in combination with a 17- or 20-oxo group occurs in the most potent inhibitors: 3β-hydroxy-5-androsten-17-one and 3β-hydroxy-5-pregnen-20-one. The replacement of the

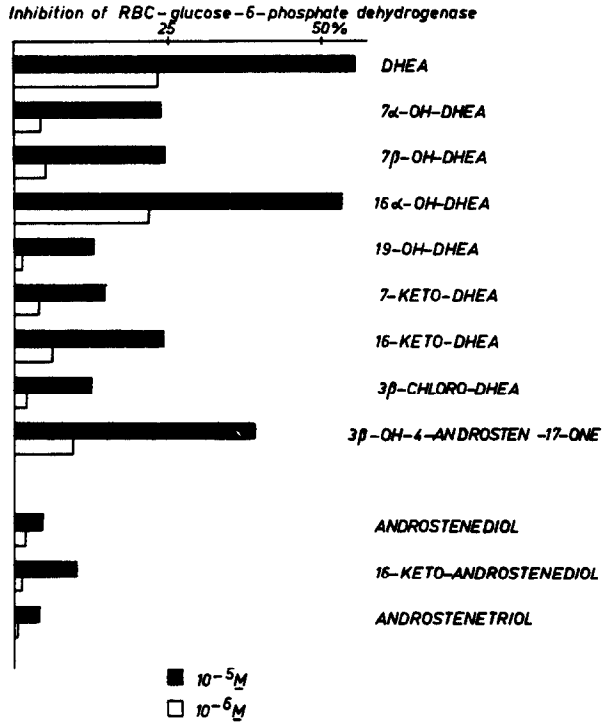

Fig. 1. Inhibition of human RBC glucose-6-phosphate dehydrogenase by 3β-substituted Δ^5- or Δ^4-C_{19}-steroids.

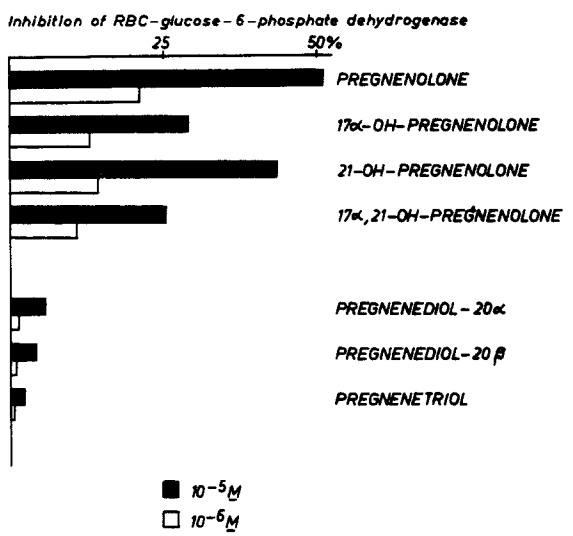

Fig. 2. Inhibition of human RBC glucose-6-phosphate dehydrogenase by 3β-hydroxy Δ^5-C_{21}-steroids.

3β-hydroxy group by a 3β-chloro group, however, caused a remarkable loss of activity. On the other hand, the location of the double bond appears to be of minor importance, as evidenced by the comparable K_i-values of 3β-hydroxy-4-andros-

ten-17-one and 3β-hydroxy-5-androsten-17-one. Likewise, a change in the conformation of the 3-hydroxy group in Δ^5-C_{19}-steroids is known to exert little influence upon the activity of such steroids in the enzyme inhibition test[1]. From such findings and the results of other authors it is suggested that a 3-hydroxy group in Δ^5-C_{19}- or C_{21}-steroids represents a second structural feature needed for outstanding inhibitory properties. Hence, the marked decrease in activity, noticed after oxidation of the 3β-hydroxy group to a 3-oxo group (3β-hydroxy-5-androsten-17-one to 4-androstene-3,17-dione) is not surprising[1].

The introduction of additional hydroxy or oxo groups at C-7 or C-19 greatly reduced the activity of 3β-hydroxy-Δ^5-C_{19}-steroids as inhibitors of G-6-PDH. Since 3β,7α- and 3β,7β-dihydroxy-5-androsten-17-one as well as 3β,19-dihydroxy-5-androsten-17-one were all found to possess only a fraction of the activity of the parent compound 3β-hydroxy-5-androsten-17-one (K_i-values between $5{\cdot}2 \times 10^{-5}$ *M* and $1{\cdot}8 \times 10^{-4}$ *M* as compared to $6{\cdot}2 \times 10^{-6}$ *M* for the latter steroid) the attachment of the steroid to the proper sites of the enzyme molecule seems to be impeded by the additional functional groups. Whether this can be attributed to mere steric hindrance or to the negative inductive effect of an oxygen function at C-7 or C-19, causing a lower electron density at C-5, remains to be clarified.

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