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EFFECTS OF DEHYDROEPIANDROSTERONE AND ITS CONJUGATES UPON THE ACTIVITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

I. INHIBITION OF HUMAN RED BLOOD CELL GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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SUMMARY

When the activity of purified glucose-6-phosphate dehydrogenase from human erythrocytes was assayed in the presence of free dehydroepiandrosterone or its different conjugates, a distinct non-competitive inhibition of the enzyme by physiological amounts of dehydroepiandrosterone sulfatide was observed. The inhibition by synthetic, biosynthetic or endogenous dehydroepiandrosterone sulfatide exceeded that produced by equimolar concentrations of the free steroid. On the basis of these results and previous data it is suggested, that under physiological conditions the regulation of glucose-6-phosphate dehydrogenase activity eventually may be mediated through a lipophile dehydroepiandrosterone sulfatide.

INTRODUCTION

IT SEEMS well established, that 3β-hydroxy-5-androsten-17-one (dehydroepiandrosterone, DHEA) represents a potent inhibitor of mammalian glucose-6-phosphate dehydrogenase (G-6-P-DH)[1–3]. DHEA sulfate, on the other hand, proved to be completely ineffective in the regular in-vitro enzyme test[4]. Under physiological conditions, a distinct inverse relationship between G-6-P-DH activity and levels of DHEA has been found[5, 6]. In the human plasma or urine, however, DHEA is known to occur almost exclusively as sulfoconjugate. Preliminary investigations concerning these discrepancies between in-vitro and in-vivo findings already indicated that human red blood cell (RBC) G-6-P-DH may be inhibited also by DHEA, esterified with diglyceride sulfuric acid or 'sulfatidic acid', e.g. by synthetic DHEA sulfatide[7]. The latter compound is assumed to be representative of lipophile steroid sulfoconjugates, as demonstrated in human plasma and tissues[8].

In continuation of the aforementioned experiments the effect of DHEA and its different conjugates upon the activity of purified G-6-P-DH from human erythrocytes was examined.

EXPERIMENTAL

Purified G-6-P-DH was prepared from 500 ml fresh human blood essentially as described by Marks *et al.*[3]. After hemolysis of carefully washed erythrocytes an ammonium sulfate precipitation at 55% saturation, followed by adsorption of proteins on calcium phosphate gel and a twofold ammonium precipitation at 50% saturation resulted in a 441-fold purification of the enzyme, as indicated by an initial specific activity of 0·234 IU/mg protein and a final specific activity of 98·5 IU/mg protein. The overall yield amounted to 13·5% of total original enzyme activity.

All assays of G-6-P-DH were performed at 25°C in 3·00 ml 0·05 *M* triethanolamine/0·005 *M* EDTA

buffer of pH 7·6 with 0·05–0·10 ml of enzyme in this buffer, 0·10 ml NADP solution and 0·05 ml glucose-6-phosphate solution of varying concentration and 0·02 ml dioxan, eventually containing DHEA or its conjugates. The following compounds were tested as potential inhibitors of purified G-6-P-DH at concentrations of $5 \times 10^{-6} M$ to $5 \times 10^{-7} M$: DHEA, DHEA glucuronoside, DHEA sulfate, synthetic DHEA sulfatide[7], biosynthetic DHEA sulfatide[9] and DHEA sulfatide, isolated from human adrenal venous blood[10]. Furthermore, a combination of DHEA sulfate and lipid material from the solvolysate of endogenous lipophile steroid sulfoconjugates was tested.

In the course of these experiments the activity of the purified enzyme was measured in the absence and presence of steroid over a wide range of substrate concentrations, using either an excess of NADP and limiting concentrations of glucose-6-phosphate or an excess of glucose-6-phosphate and limiting concentrations of NADP[1, 11].

RESULTS

The K_M-value of the 441-fold purified human RBC G-6-PDH with respect to glucose-6-phosphate was found to be $4{\cdot}1 \times 10^{-5} M$. The activity of contaminating 6-phospho-gluconate dehydrogenase, assayed by the method of Glock and McLean[12] approximated 0·09% of G-6-P-DH activity in NADP reduction. The reciprocal plots of the enzyme activity in the presence of a constant amount of steroid or steroid conjugate and excess NADP and limiting concentrations of glucose-6-phosphate are presented in Figs. 1 and 2. While Fig. 1 depicts the data obtained with free DHEA, its glucuronoside, sulfate or synthetic sulfatide, Fig. 2 shows the results of similar experiments with the DHEA sulfatides of various sources, as well as with the mixture of DHEA sulfate and plasma lipids. In Fig. 3 the effect of DHEA or its different conjugates upon enzyme activity in presence of excess glucose-6-phosphate and limiting con-

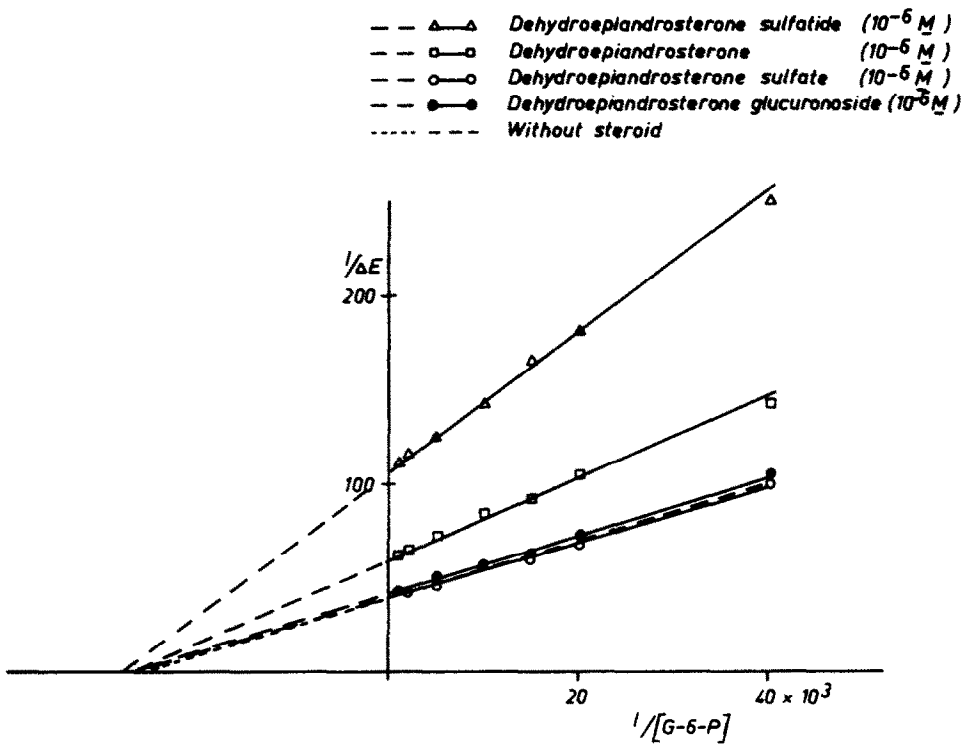

Fig. 1. Inhibition of human RBC G-6-P-DH by DHEA or its conjugates in the presence of excess NADP and limiting concentrations of glucose-6-phosphate.

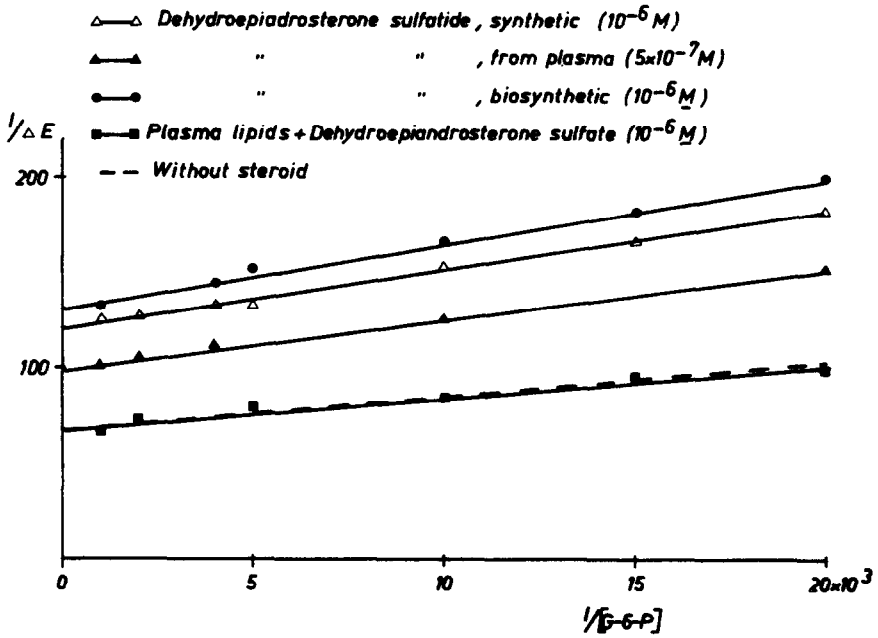

Fig. 2. Inhibition of human RBC G-6-P-DH by DHEA sulfatide from different origin in the presence of excess NADP and limiting concentrations of glucose-6-phosphate.

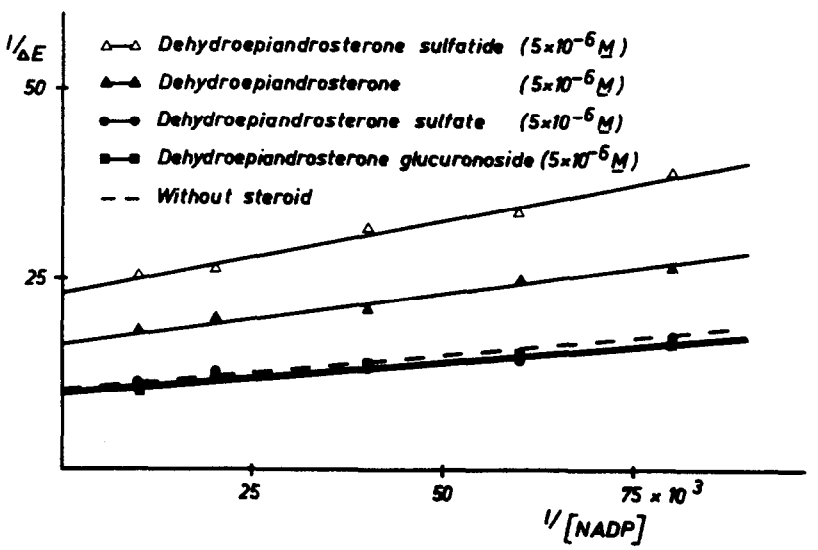

Fig. 3. Inhibition of human RBC G-6-P-DH by DHEA or its conjugates in the presence of excess glucose-6-phosphate and limiting concentrations of NADP.

centrations of NADP is demonstrated. Finally, the determination of the inhibitor constant by the method of Hunter and Downs [13] is to be seen in Fig. 4.

DISCUSSION

From Figs. 1–4 it becomes quite evident, that the inhibition of purified human RBC G-6-P-DH by DHEA is not limited to the free steroid as hitherto assumed

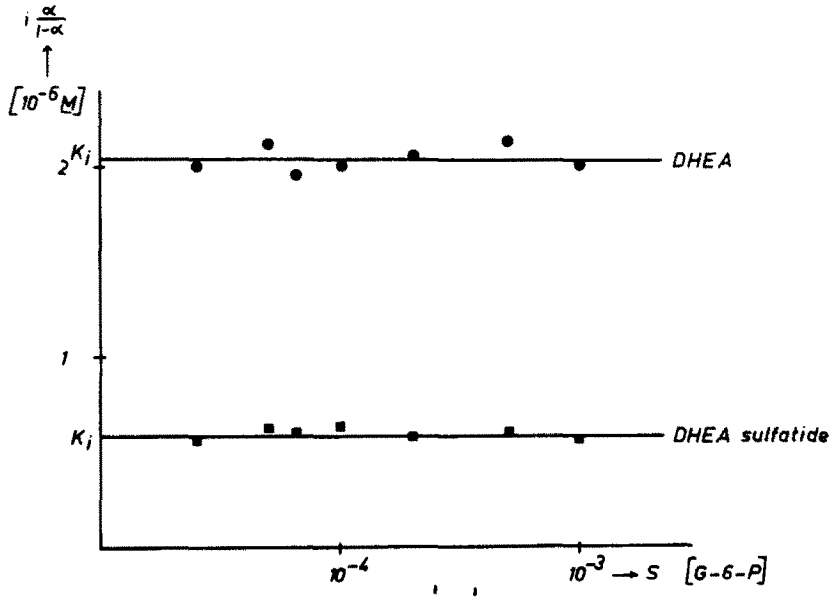

Fig. 4. Graphical determination of K_i for DHEA and synthetic DHEA sulfatide by the method of Hunter and Downs[13].

[1, 4]. In fact, at a concentration of $5 \times 10^{-6} M$ the synthetic, biosynthetic or endogenous DHEA sulfatide proved to be nearly twice as effective an inhibitor as the free compound (51 vs. 33% inhibition (for the synthetic conjugate)). This concentration, for instance, lies well below normal levels of sulfoconjugated DHEA e.g. DHEA sulfatide in human plasma, which range between 40 and 250 μg/100 ml or $1{\cdot}4$–$8{\cdot}7 \times 10^{-6} M$[14]. Variations in the extent of inhibition, produced by the different DHEA sulfatides, may be attributed to the admixture of other steroid sulfatides in the biosynthetic or endogenous material. The present results seem to favor the hypothesis, that under physiological conditions the endogenous DHEA sulfatide may control the activity of G-6-P-DH, and provide an explanation for seemingly controversial evidence furnished by in-vitro or in-vivo experiments[1, 4–6].

Since the reciprocal plots of enzyme activity in the absence or presence of constant amounts of inhibitor and limiting concentrations of glucose-6-phosphate or NADP yielded straight lines intersecting on the base line, the inhibition may be classified as non-competitive[11, 13, 15]. Using the method of Hunter and Downs[13], the plot of substrate concentration s versus inhibitor concentration i multiplied by $\alpha/(1-\alpha)$ (α representing the quotient v_i/v) resulted in a straight line parallel to the base line and indicated an inhibitor constant K_i of $0{\cdot}6 \times 10^{-6} M$ for synthetic DHEA sulfatide.

Upon preincubation of the enzyme with DHEA or its sulfatide at a concentration of $5 \times 10^{-6} M$ in the presence or absence of NADP and/or glucose-6-phosphate no difference in inhibition could be observed. Therefore, the degree of inhibition by free DHEA or its sulfatide should not be related to the order of addition of the reactants. Furthermore, after incubation of the enzyme with free DHEA or its sulfatide and NADP or NADPH for 15 min at 25°C no substantial change in nucleotide concentration was noticed spectrophotometrically, thus excluding

a NADP linked oxidation or reduction of the steroid moiety. However, substitution of non-labeled steroid by 5×10^{-6} *M* concentrations of 7α-^{3}H-DHEA or its different sulfoconjugates led to the detection of minor amounts of steroid metabolites.

From the data of Marks and Banks[1], obtained with the same purified G-6-P-DH (K_M for glucose-6-phosphate $= 3{\cdot}5 \times 10^{-5}$ *M*) it was concluded, that the inhibition of the enzyme by various steroids is dependant upon the presence of an oxo group in C-17 or C-20, whereas additional hydroxy or oxo groups in C-3 and double bonds at C-4[5] or C-5[6] were considered to be of little effect with regard to the inhibitory action of the steroid. Obviously, such a concept is hardly compatible with the complete lack of enzyme inhibition by 10^{-6} *M* concentrations of 4-pregnene-3,20-dione (progesterone), 3-hydroxy-1,3,5-estratriene-17-one (estrone) or DHEA sulfate and glucuronoside, nor with the significantly greater inhibition of the enzyme by DHEA sulfatide as compared to that induced by the free DHEA. Instead, these findings suggest a participation of the 3-hydroxy (or 3-oxo) group in the interaction between steroid and enzyme, leading to the non-competitive inhibition of the latter. Together with an oxo group, preferably in the C-17 position, the oxygen function at C-3—expecially an equatorial 3β-hydroxy group—may confer a certain polarity to the steroid molecule which is required for its access to the proper sites of the enzyme molecule in order to change its conformation. Such negative charges, as encountered in the anions of DHEA sulfate or glucuronoside, as well as in the phenolic hydroxy group of estrone, apparently prevent any attachment of the steroid to the protein molecule. The transformation of the steroid sulfate into its diglyceride ester or 'sulfatide' removes the negative charge and provides the essential lipophile character of the steroid inhibitor.

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