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INHIBITION OF HUMAN ERYTHROCYTE GLUCOSE 6-PHOSPHATE DEHYDROGENASE ACTIVITY BY DEHYDROEPIANDROSTERONE AND RELATED STEROIDS

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ABSTRACT

The inhibitory effects of several steroids on G6PD activity using intact erythrocytes are reported. Incubation of whole blood with dehydroepiandrosterone (DHEA) resulted in 42% and 12% inhibition in the enzyme activity in the presence and absence of oxygen, respectively. Addition of epinephrine and/or aminophylline into the incubation medium caused further enzyme inhibition suggesting a possible involvement of cAMP in the mechanism by which G6PD activity is inhibited in the system used. The activity of the enzyme was also inhibited by testosterone propionate (11 %), progesterone (25%), and estradiol (15%) at their physiological concentrations. The data obtained provides further evidence for the possible regulatory effects of steroids, particulary DHEA, on G6PD activity.

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INTRODUCTION

Glucose6-phosphate dehydrogenase (EC.1.1.1.49, G6PD), is a key enzyme which catalyzes the first reaction of the pentose phosphate pathway, providing reducing equivalents (NADPH) for a variety of biosynthetic reactions. Hormonal regulation of this enzyme, in a number of mammalian tissues, has been reported by several investigators.[1-6] Although steroids were found to be potent inhibitors of G6PD,[7] the physiological significance of this inhibition is not yet certain. An interrelationship between dehydroepiandrosterone (DHEA), G6PD activity, and cyclic AMP (cAMP) levels in normal and diseased subjects has also been reported.[8,9]

In the present study the regulatory effects of a number of steroids on human erythrocyte G6PD have been investigated, and the possible relationship between DHEA and cAMP/G6PD system is further discussed.

MATERIALS AND METHODS

Reagents

Glucose 6-phosphate, $NADP^+$, testosterone, testosterone propionate, DHEA, estradiol, progesterone, epinephrine and aminophylline were obtained from Sigma Co. (U.S.A). All other chemicals were reagent grade.

Incubation conditions

The effects of different steroids were studied by adding them to the whole human blood incubated at 37°C. All experiments were performed in the presence of oxygen (1 lit/min/vial), unless otherwise specified. Each steroid was dissolved in ethanol and introduced to a vial in such amount as to obtain designated concentrations, and evaporated to dryness under a stream of nitrogen. Human blood samples were collected on ACD (Acid, Citrate, Dextrose) (3ml) and were added into the vials and incubated for different time intervals.

During the incubation period, water lost by evaporation was replaced with prewarmed water at frequent regular intervals.

When the effects of epinephrine or aminophylline were studied, aliquots (5 μl) of fresh aqueous solutions of epinephrine and/or aminophylline were added to the appropriate incubation mixture to give the final concentrations of 0.5 mM and 7 mM, respectively.

Control samples, using blood alone were always incubated in parallel with the experimental vials.

At the end of the incubation time, red blood cells of each sample were separated by low speed centrifugation, washed three times with 0.9% NaCl and hemolysed in 5 mM phosphate buffer, pH 7.7, containing 10 μM $NADP^+$, 7 mM ß-mercaptoethanol and 2.7 mM EDTA, according to the WHO-recommended method.[10] The hemolysate was then centrifuged at 28,000 g for 20 min at 4°C, using a Sorvall Centrifuge, Model RC-50; the supernatant fraction was removed and kept at 0-4°C prior to the enzyme assay.

Enzyme assay

The activity of G6PD was measured as described by Cohen, et. al.[11] using a Beckman spectrophotometer, model 25, with a thermostated cell compartment maintained at 30°C. One unit of enzyme activity was taken as the number of μmoles of $NADP^+$ reduced per minute. Protein concentration was determined by the method of Lowry, et al.[12]

RESULTS

DHEA inhibited G6PD activity in either the presence or absence of oxygen (Fig.1). Higher degrees of inhibition were observed at all DHEA concentrations in the presence of oxygen than in its absence. At physiological concentration of hormone (5.5 μm), the percentages of inhibition were 42 ± 2% and 12 ± 4% in the presence and absence of oxygen, respectively. Oxygen alone was also shown to inhibit the enzyme activity by 30 ± 5%. Time-course experiments showed that the maximum inhibition by DHEA occurred after one hour incubation. When DHEA at physiological concentration was directly added to the assay mixture, the activity of the enzyme decreased by 40%.

The enzyme appeared to be more inhibited when epinephrine and/or aminophylline together with DHEA were present in the medium (Table I).

Testosterone propionate at the concentration of 30 nm decreased the enzyme activity by 11 ± 0.5% ($P < 0.01$). When the hormone concentration rose up to 1500 nm no further significant enzyme inhibition was observed. Free testosterone, however, made no change in G6PD activity under the same conditions. At physiological concentrations of progesterone (3.5 nm) and estradiol (1.8 nm) the enzyme was inhibited by 25 ±2% and 15±1%, respectively, but no significant increase was observed in the degree of inhibition at higher concentrations (Table II).

Table I. The effects of DHEA, epinephrine and aminophylline on G6PD inhibition.

Samples	% Inhibition
EHEA (0.006 mM)	42 ± 2
Epinephrine (0.5 mM)	18 ± 0.8
Aminophylline (7 mM)	24 ± 1.2
DHEA + epinephrine	62 ± 2.7
DHEA + aminophylline	54 ± 3.0
Aminophylline + epinephrine	27 ± 2.2
DHEA + aminophylline + epinephrine	63 ± 5.0

Values represent mean ± SD of at least four experiments. For details see text.

DISCUSSION

It has already been reported that some steroids could inhibit the activity of G6PD obtained from a number of sources.[7] In the present study the inhibitory effects of several steroids on G6PD activity were shown using intact erythrocytes.

No change in G6PD activity was observed when erythrocytes were washed with normal saline and resuspended in either plasma or normal saline and then

Table II. Percent inhibition of G6PD activity in the presence of different concentrations of testosterone propionate, progesterone, and estradiol.

Hormone	Concentrations (nM)	% Inhibition
Testosterone Propionate:		
	30	11 ± 0.5
	300	15 ± 1.0
	750	13 ± 0.9
	1500	16 ± 0.9
Progesterone:		
	3.2	25 ± 2.0
	54	27 ± 0.5
	108	29 ± 1.0
	1080	27 ± 0.6
	2700	25 ± 1.5
	5400	27 ± 1.0
Estradiol:		
	1.8	15 ± 1.0
	18	12 ± 3.0
	27	15 ± 1.5
	90	20 ± 2.5
	135	19 ± 1.0
	180	20 ± 2.0

Each value is the mean ± SD of at least four experiments. For details see text.

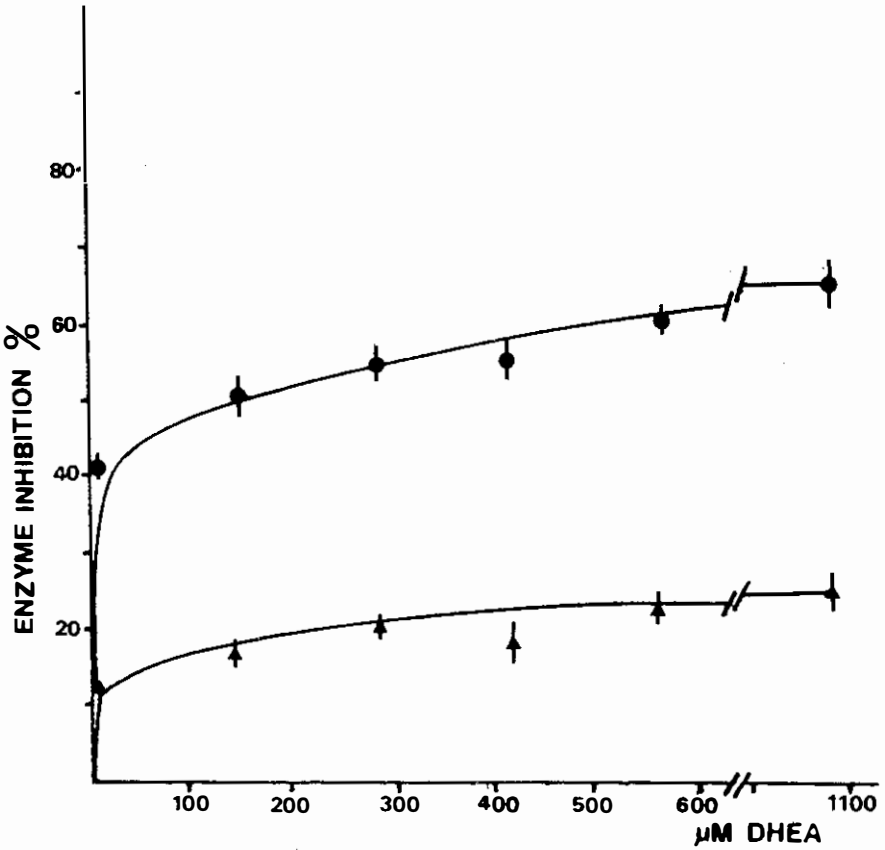

Fig. 1. The effect of different concentrations of DHEA on G6PD inhibition in the presence (●) and absence (▲) of oxygen (for details see text).

incubated with DHEA in the presence or absence of oxygen. Incubation of whole blood, however, with DHEA led to the enzyme inhibition (Fig. 1), indicating that peripheral constituents on the membrane are posibly essential for DHEA action.

The inhibitory effect of oxygen on G6PD activity remains to be explained. However, the possibility of direct oxidation of protein reactive groups, which are necessary for the enzyme activity, by oxygen exists. A relationship between G6PD activity and hypoxia, hyperoxia,[13] and carbon dioxide,[14] has been also reported by other investigators.

Because steroid inhibitors have K_i's below 1 μM,[15] and DHEA at its physiological concentration (5 μM) and at much lower concentrations (Fig. 1) exerts its inhibitory effects, the *in vivo* regulatory role of this steroid is likely.

Our results indicate that there is apparently a relationship between cAMP production and G6PD activity in erythrocytes (Table I). When cAMP levels in the cell is elevated by either epinephrine, which activates its production, or aminophylline, which inhibits its breakdown, there is an increase in the degree of the enzyme inhibition. There are several contradictory reports concerning the relationship between DHEA and cAMP/G6PD system. Oertel, et. al.[9] demonstrated that DHEA and DHEA-sulfatide may stimulate cAMP-phosphodiesterase. Glucose 6-phosphate, on the other hand, is reported to inhibit the activity of this enzyme.[16] It has also been shown that the intracellular concentration of cAMP is decreased in the presence of DHEA sulfatide.[9] However, decreased levels of plasma DHEA in hyperlipemic, psoriatic, psychotic, or menopausal patients have been shown to be accompanied by increased erythrocyte G6PD activity and decreased cAMP concentrations in plasma and erythrocytes.[8] Cyclic-AMP itself is found to inhibit G6PD competitively with respect to glucose 6-phosphate.[17] It might be possible that intracellular elevation of cAMP, produced by either epinephrine or aminophylline in our study, leads to direct inhibition of G6PD which is added to the DHEA inhibitory effect. However, the interrelationship between cAMP and DHEA in inhibiting G6PD needs further investigations.

Raineri and Levy[15] found that inhibition of G6PD by steroids requires carbonyl group at C-17 for androstanes and esteranes and at C-20 for pregnanes. The

inhibition of G6PD in intact erythrocytes by DHEA and progesterone (Fig. 1, Table II) confirmed this finding. However, testosterone propionate and estradiol which lack these carbonyl group also caused G6PD inhibition (Table II) suggesting that probably other mechanisms are involved.

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