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THE INHIBITORY EFFECT OF DEHYDROEPIANDROSTERONE ON THE ACTIVITY AND ISOENZYME PATTERNS OF LIVER GLUCOSE 6-PHOSPHATE DEHYDROGENASE DURING DIFFERENT STAGES OF GROWTH

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Abstract

The effect of dehydroepiandrosterone (DHEA) on the activity and isoenzyme patterns of liver glucose 6- phosphate dehydrogenase (G6PD) was investigated in rats during different stages of growth. G6PD activity of mature rats was less than those of the immature (43%) and newborn (35%) animals. DHEA at 10^{-4} M) inhibited G6PD activity of the mature, immature and newborn rats by 79%, 39%, and 88%, respectively. Digitonin (0.02%) abolished the inhibitory effect of DHEA in all three groups of rats with an additional 22% increase in the enzyme activity in only the mature animals. Gel electrophoresis band patterns of G6PD showed four active bands (I, II, III, IV) for the newborn and immature rats. In mature rats band IV was absent but appeared in the presence of digitonin. When electrophoresis was done in the presence of DHEA (10^{-4} M) band IV of the immature and newborn animals disappeared and the activity of band I for all animals was partially inhibited with no change in the activities of bands II and III. The data provide further evidence for the possible regulatory role of DHEA on G6PD isoenzyme activities.

Introduction

Glucose 6- phosphate dehydrogenase (EC 1. 1. 1. 43) catalyzes the first reaction of pentose phosphate pathway providing NADPH necessary for many biosynthetic reactions. Several steroids inhibit the activities of G6PD's from different sources [for review see ref. 1]. DHEA, and important adrenal androgen, has been shown to potently inhibit G6PD activity in a number of mamalian tissues [2-4]. Administration of DHEA orally to man [5] and dietary to male rats [6] also inhibited G6PD activity. The inhibitory effect of DHEA on the isoenzymes of liver [7] and heart [8] G6PD's have been reported. It appears that different forms of G6PD show different sensitivities toward DHEA. Hepatic G6PD of obese rats is more sensitive to DHEA than the enzyme from lean rats [9]. In rats, hepatic G6PD activity has been found to be age-dependent [10] and following physical activities, where there is an increase in the concentration of steroid hormones [11], the enzyme activity was decreased with the effect being isoenzyme- specific [12]. It seems likely that variations in the steroid hormones may be responsible for the changes observed in G6PD activity.

The present study was, therefore, undertaken to examine the effects of DHEA on the total enzyme activity and on the isoenzyme patterns of rat liver G6PD during different stages of growth during which, the steroid hormone concentration also varies.

Key words: Hormonal regulation of G6PD isoenzymes

Materials and Methods

Wistar male rats were obtained from Pasteur Institute (Tehran). Three groups of newborn (10- days), immature (1- month) and mature (4- months) rats were kept in a room with 12 h periods of light and dark. The animals had access to a 41 B diet [13] and drinking water. The rats were sacrificed by decapitation in groups of four of each age.

Determination of G6PD activity: The liver of each rat was removed, washed with saline, homogenized and the enzyme activity measured in the homogenate as described by Lopez and Rene [7]. The assay mixture was 1 ml of 120 mM Tris- HCl pH 8 containing 0.2 mM

glucose 6- phosphate, 0.9 mM $NADP^+$, 10.4 mM $MgCl_2$ and appropriate amount of the enzyme solution. DHEA was dissolved in 10% ethanol and added just before addition of the enzyme. Ethanol (10%) alone had no effect on the enzyme activity. One enzyme unit was taken as the amount of enzyme required for production of 1 μmole of NADPH per minute. The reaction was monitored by measuring the change in optical density at 340 nm at 37°C using a Beckman spectrophotometer Model 50.

Gel electrophoresis: Disc gel electrophoresis was performed on 5% gel as described by Gabriel [14]. Samples of the liver homogenate were applied (50 μl) on gel columns and after electrophoresis the gels were stained in 0.1 M Tris- HCl buffer pH 8.6 containing 2 mM glucose 6- phosphate, 0.5 mM $NADP^+$, 0.07 mM phenazine methosulfate and 0.5 mM p- iodonitrotetrazolium violet [7]. Protein concentration was measured by the method of Lowry et. al. [15].

Results

The activity of hepatic G6PD during different stages of growth is shown in Table 1. The specific enzyme activity of the mature rats was less than those of the immature (43%) and newborn (35%) animals. DHEA at 10^{-4} M concentration inhibited G6PD activities of newborn, immature and mature rats by 88%,89%, and 70%, respectively. The effect was also observed at lower hormone concentration (10^{-5} M). Addition of digitonin (0.02%) together with DHEA in the assay mixture abolished the inhibitory effect of the hormone in all three groups of rats with an additional 22% increase in the enzyme activity of the mature animals only.

Disc gel electrophoresis experiments (Table 2) showed four active bands (I, II, III, IV) for the newborn and immature rats. In the isoenzyme pattern of the mature rats, however, band IV did not appear. Addition of DHEA (10^{-4} M) in the homogenate and subsequent electrophoresis inhibited the activities of bands I (about 80%) of all three groups of rats and completely abolished bands IV of newborn and immature animals. Bands II and III, however, were not affected. Neither digitonon nor DHEA altered relative mobilities of the active bands. It seem that DHEA exhibits its inhibitory effect mainly on bands I and IV.

Discussion

In vitro inhibition of G6PD activity of rat liver by DHEA has been previously reported [7]. The age-dependent variations in hepatic G6PD activity observed in this study further supports the idea that an increase in the endogenous steroid levels such as DHEA could lead to the decrease in G6PD activity [8]. This is also shown by *in vitro* finding that G6PD of mature rats was less sensitive to DHEA inhibition than those of immature and newborn animals. Digitonin restored the enzyme activities of all three groups of animals, inhibited by DHEA *in vitro*, and further increased G6PD activity of only mature rats (Table 1).

TABLE 1:
The effect of dehydroepiandroterone (DHEA) on the activity of rat liver G6PD during different stages of growth.

Added	Newborn		Immature		Mature	
	S.A.	%	S.A.	%	S.A.	%
None	17.12+0.81	100	19.36+1.31	100	11.1+1.11**	100
+DHEA (10^{-5}M)	5.61+0.47*	33	6.82+0.53*	35	5.73+0.8*	48
+DHEA(10^{-4}M)	2.06+0.33*	12	1.95+0.35*	11	2.88+0.44*	21
+DHEA(10^{-4}M)+ Digitonin(0.02%)	17.0+0.7	99	19.86+0.8	102	13.47+1.6*	122
+Digitonin(0.02)	17.1+0.79	100	19.2+1.21	99	13.36+1.4	120

The values are mean ± s. d. of three different experiments. Untreated samples in each group were taken as 100%.
★, ★★; Significantly different from controls ($p < 0.01$)
(★★, vs younger rats: ★, vs correponding controls (None))
S. A., Specific enzyme activity (m. i. u Protein)

This may be due to the interaction of digitonin with the endogenous steroids in the liver homogenate of the mature rats.

Administration of DHEA dietary to male rats [6] and orally to man [5] also decreased G6PD activity. Cleary and Zisk [9], however, have reported that although dietary administration of this hormone decreases hepatic G6PD activity of obese rats, it increases the enzyme activity of the lean animals.

It appears from gel electrophoresis experiments (Table 2) that DHEA has isoenzyme specificity and exerts its effects primarily on band IV and partly on band I leaving bands II and III unaffected. Lopez and Rene [7].have shown that DHEA at 10^{-5} M concentration uniformly inhibits all four active bands of hepatic G6PD but at higher concentration (10^{-4} M) abolishes band D (fastest band). In rats, stress induced by swimming decreased liver G6PD activity relating mainly to the decrease in the levels of isoenzymes II and IV but 6 h after the stress liver enzyme activity has been above normal due to increase in isoenzymes II and IV [12]. Polyacrylamide gel electrophoresis of rat heart G6PD has shown three isoenzymes (A, B, C) from which band C is mainly inhibited by DHEA [8].

These findings plus age- dependent changes observed in the serum DHEA and DHEA- sulfate levels of both men and women [16, 17] and in the rat G6PD activity [10] provide evidence for the possible regulatory effects of DHEA on G6PD activity.

TABLE 2:
Disc gel electrophoresis of hepatic G6PD from newborn, immature and mature male rats in the presence and absence of DHEA.

RATS & ADDED	RELATIVE MOBILITY VALUES OF ACTIVE BANDS*			
	I	II	III	IV
NEWBORN:				
None	0.385+0.007	0.290+0.005	0.210+0.002	0.134+0.012
DHEA	0.392+0.004	0.280+0.001	0.214+0.002	NA
DHEA+ Digitonin	0.379+0.008	0.293+0.003	0.216+0.002	0.141+0.009
IMMATURE:				
None	0.370+0.001	0.281+0.009	0.210+0.006	0.162+0.007
DHEA	0.383+0.008	0.280+0.009	0.212+0.004	NA
DHEA+ Digitonin	0.370+0.007	0.282+0.007	0.201+0.002	0.150+0.007
MATURE:				
None	0.384+0.003	0.287+0.004	0.197+0.004	NA
DHEA	0.381+0.006	0.279+0.009	0.196+0.006	NA
DHEA+ Digitonin	0.379+0.007	0.281+0.005	0.20+0.001	0.130+0.005

*, Activities of bands I and IV were inhibited by DHEA by 80% and 100 %, respectively. Values represents mean ± s.d. of 5 to 7 experiments. NA, not appeared. For details see text.

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