AGE-DEPENDENT AND ISOENZYME SPECIFIC INHIBITION OF HEART GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY DEHYDROEPIANDROSTERONE

M. S. SETCHENSKA, E. M. RUSSANOV, J. G. VASSILEVA-POPOVA
Central Laboratory of Biophysics, Bulgarian Academy of Sciences, Sofia 13, Bulgaria

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1. Introduction

Glucose 6-phosphate dehydrogenase (EC 1.1.1.43) is the rate-limiting enzyme in the hexosemonophosphate pathway and plays an important role in lipogenesis and steroid biosynthesis. The enzyme is inhibited by steroid hormones, as was first reported by Marks and Banks [1] and by McKerns et al. [2]. It is also known that glucose 6-phosphate dehydrogenase (G-6-PDH) from various mammalian sources is sensitive to steroids but not the enzyme from spinach and yeast [1]. Levy [3] found that dehydroepiandrosterone inhibited the NADP- but not the NAD-linked G-6-PDH isolated from mammary glands of lactating rats. Urea, glycerol, high pH and increased temperature were found to reverse the inhibition by dehydroepiandrosterone, suggesting alterations in the three-dimensional structure of the enzyme and the removal of the steroid-binding site from the proximity of the active centre. Human testicular [4], ovarian [5] and placental [6] G-6-PDH are also inhibited by different steroid hormones as well as by their derivatives, analogues and conjugates, dehydroepiandrosterone (DHEA) being the most potent inhibitor in vitro. When studying the specificity of steroid interaction with mammary G-6-PDH, Raineri and Levy [7] found that the inhibition requires the presence of a keto group at C_{17} for androstanes and estranes and at C_{20} for pregnanes, it is non-competitive with respect to both glucose 6-phosphate and NADP and a hydrophobic interaction between the enzyme and steroid hormones has been assumed.

As regards to the isoenzyme specificity for steroid hormone inhibition with G-6-PDH from rat adipose tissue (which reacts not only with NADP but also with NAD), the activities of all NADP- and NAD-linked G-6-PDH isoenzymes were markedly reduced by dehydroepiandrosterone [8]. With rat liver G-6-PDH, Lopez and Rene [9] have shown that at $5 \cdot 10^{-5}$ M dehydroepaindrosterone uniformly inhibits all four isoenzymes with complete disappearance of band D at the higher concentration of 10^{-4} M. Hori and Matsui [10] have reported specific isoenzyme inhibition of G-6-PDH by dehydroepiandrosterone.

The object of the present study was to investigate the age-dependent sensitivity of rat heart G-6-PDH to dehydroepiandrosterone (Δ^5-androstane-3β-01-17-one) as well as to look for a pattern of specific isoenzyme inhibition. In this paper we report that the heart enzyme from young rats was more sensitive to inhibition by DHEA than G-6-PDH from older animals, and that this inhibition is abolished by low concentrations of digitonin. Elevation of endogenous circulating steroid levels by exhaustive exercise also caused an inhibition of enzyme activity. Electrophoretic analysis of G-6-PDH isoenzymes showed that DHEA selectively inhibited one of three isoenzymes.

2. Materials and methods

Two groups of six male 4 weeks-old (immature) and 3 months-old (adult) rats were used. A 10 000 *g* supernatant obtained from heart homogenate was used as the enzyme preparation and the reaction mixture for determination of the total G-6-PDH activity consisted of 0.1 M Tris–HCl buffer, pH 7.6, 0.5 mM NADP and the enzyme, the reaction being started by the addition of 3.5 mM glucose 6-phosphate. Dehydroepiandrosterone was dissolved in dioxane

and added just before the enzyme. Dioxane alone (0.7% of the final assay volume) had no effect on the enzyme activity. Changes in optical density at 340 nm were measured at 37°C in a cell with 1 cm light path at 30 sec intervals. Protein was determined by the Lowry method. The specific activity of the enzyme was expressed in μmole NADP reduced per min per mg protein. The results presented were obtained from six experiments and were statistically significant.

Isoenzyme patterns were determined by the standard Davis procedure [11] for polyacrylamide disc electrophoresis with Tris–glycine electrode buffer, pH 8.3, at a constant current of 4 mA per tube. On each gel 500 μg protein were layered and, after electrophoresis, the gels were stained for enzyme activity at 37°C for 60 min in the following reaction mixture: 95 μmoles Tris–HCl buffer, pH 7.6, 0.9 μmoles NADP, 1 mmol $MgCl_2$, 0.04 mg PMS, 1 mg nitrotetrazolium blue and 8 mmoles glucose 6-phosphate.

3. Results and discussion

Table 1 shows that the total activity of heart G-6-PDH from immature rats is about 25% higher than that from mature ones which may reflect an in vivo inhibition of G-6-PDH by the increasing endogenous steroid hormones as the rats mature. The enzyme from immature rats is also more sensitive to dehydroepiandrosterone, a 50% inhibition being obtained at 10^{-6} M with the enzyme from young rats whereas a 50% inhibition of the enzyme from old rats required a concentration of 10^{-5} M dehydroepiandrosterone. Complete inhibition of the enzyme was obtained at 10^{-5} M and 10^{-4} M for the immature and adult rats, respectively. These results further showed that rat heart G-6-PDH is more sensitive to steroid inhibition than rat liver G-6-PDH is more sensitive to steroid inhibition than rat liver G-6-PDH, the latter being inhibited by 50% with $5 \cdot 10^{-5}$ M dehydroepiandrosterone.

The addition of digitonin (0.02% final concentration) to the reaction mixture abolished dehydroepiandrosterone inhibition and at the same time increased G-6-PDH activity from mature rats, but not young rats, by about 30% (table 1). It is known that digitonin can react with the 3-hydroxy group of steroids forming an insoluble complex as well as affect hydrophobic bonds. The increase in G-6-PDH activity from mature rats by the addition of digitonin can be explained by digitonin interacting with endogenous steroids in heart homogenates since it did not increase the activity of G-6-PDH from immature rats.

The objection has been made that the endogenous concentrations of steroid hormones are two to three orders of magnitude below the concentrations required for the in vitro inhibition of G-6-PDH [12]. We

Table 1
Inhibitory effect of dehydroepiandrosterone (DHEA) on the total activity of heart glucose-6-phosphate dehydrogenase from immature and mature male rats

Addition to the assay mixture	Immature rats		Mature rats	
	Specific Activity*	% Activity**	Specific Activity*	% Activity**
None	0.018	100.0	0.015	100.0
+ 10^{-6} M DHEA	0.010	55.5	0.012	80.0
+ $5 \cdot 10^{-6}$ M DHEA	0.006	33.3	–	–
+ 10^{-5} M DHEA	no activity	0.0	0.008	53.3
+ $5 \cdot 10^{-5}$ M DHEA	–	–	0.005	33.3
+ 10^{-4} M DHEA	–	–	no activity	0.0
+ 10^{-6} M DHEA + Digitonin	0.018	100.0	–	–
+ 10^{-5} M DHEA + Digitonin	–	–	0.020	133.3
+ Digitonin only	0.018	100.0	0.020	133.3

* μMoles NADP reduced/min/mg protein

** The values of untreated control samples in each group of mature (3 months old) and immature (4 weeks old) male rats are taken as 100%.

Table 2
Effect of exhaustive swimming on the total activity of heart glucose-6-phosphate dehydrogenase from immature and mature rats

Experimental conditions	Immature rats		Mature rats	
	Specific Activity*	% Activity**	Specific Activity*	% Activity**
1. Before swimming	0.018	128.5	0.014	100.0
2. After swimming	0.018	128.5	0.010	71.4
3. After swimming + digitonin to the assay mixture	0.018	128.5	0.017	121.4

* μMoles NADP reduced/min/mg protein
** The value for control mature rats is taken as 100%.

therefore performed an experiment to test the possibility of G-6-PDH inhibition by increasing the endogenous level of steroid hormones in the animal. Since it is known that after intensive muscular exercise there is an increase in the level of steroid hormones [13], we decided to measure the G-6-PDH activity in the hearts of mature and immature rats after physical exercise: exhaustive swimming for 3–4 hr. It was found that after swimming the enzyme activity was reduced in mature rats, but not in the immature animals (table 2). The addition of digitonin to the assay mixure increased the activity of G-6-PDH from mature rats after swimming, but not in immature ones after similar treatment.

Raineri and Levy [7] suggested that the inhibitory steroids were bound to a hydrophobic pocket in the G-6-PDH molecule which had the approximate dimensions of 15 X 8 X 6 Å, one side of which appeared to be virtually planar under optimum conditions of binding since the best inhibitors tested were characterised by a large planar surface on the α-side which played the dominant role in binding the steroid to the enzyme. Our results with digitonin support the idea of hydrophobic interactions involved in the loose binding of steroid to the enzyme. Levy [3] has shown that urea, glycerol, high pH and temperature reversed dehydroepiandrosterone inhibition which was interpreted as a result of an alteration in the three-dimensional structure of the enzyme. Our experiments with digitonin suggest that no conformational change was necessary for recovering the enzyme activity inhibited, but that only the damaging of hydrophobic bonds between steroid hormone and G-6-PDH molecule sufficed to reverse the inhibition.

From the electrophoretic isoenzyme patterns shown in fig. 1, it was evident that rat heart G-6-PDH had three NADP-linked isoenzymes: bands A, B and C with relative electrophoretic mobilities of 0.13, 0.21 and 0.33, respectively. The most pronounced difference between the zymograms of immature and mature rats is the higher activity of band C of G-6-PDH from immature rats. Before staining, some gels were pre-incubated at room temperature for 15 min in 0.1 M Tris–HCl buffer, pH 7.6, containing dehydroepian-

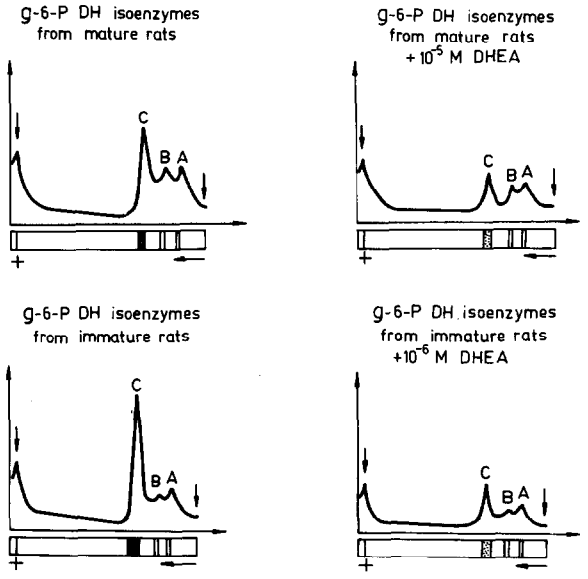

Fig. 1. Isoenzyme patterns for heart glucose 6-phosphate (G-6-PDH) dehydrogenase from mature and immature rats and the specific inhibition by dehydroepiandrosterone (DHEA) of isoenzyme band C.

drosterone in that concentration which caused 50% inhibition of the total G-6-PDH activity, i.e. 10^{-5} M for mature and 10^{-6} M for immature rats. After such treatment the activity of band C isoenzyme was markedly inhibited while the activities of bands A and B were only slightly affected. Thus, the inhibitory effect of dehydroepiandrosterone on heart G-6-PDH is manifested mainly through the selective decrease of the band C isoenzyme activity. The lower activity of isoenzyme C from mature rats is probably due to its specific in vivo inhibition by the higher levels of endogenous steroid hormones. This selective inhibitory effect of dehydroepiandrosterone on G-6-PDH isoenzymes is a novel finding of special interest as little is known in general about the regulation of isoenzymes in mammalian tissues.

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