Journal of Steroid Biochemistry, 1971. Vol. 2, pp. 289–292. Pergamon Press. Printed in Great Britain

INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY STEROIDS—III

EFFECTS OF DEHYDROEPIANDROSTERONE, ITS CONJUGATES AND DERIVATIVES UPON THE ACTIVITY OF HUMAN PLACENTAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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(*Received* 23 *March* 1971)

SUMMARY

By incubation of purified human placental glucose-6-phosphate dehydrogenase (G-6-PDH) with DHA, its conjugates or other derivatives, it could be demonstrated that DHA sulfate, phosphate and glucuronoside do not inhibit this enzyme. Conversely, DHA sulfatide proved to be extremely active in the enzyme inhibition test, whereas lipophile DHA derivatives like DHA laurate or stearate showed no activity. The addition of proteins or lipids to incubates with DHA sulfate remained without effect. Since under physiological conditions a distinct relationship has been established between sulfoconjugated DHA and human G-6-PDH activity, the endogenous sulfoconjugate of DHA may be represented by the lipophile DHA sulfatide rather than by the anionic DHA sulfate.

IN PREVIOUS communications the effect of various C_{19}- and C_{21}-steroids upon the activity of human glucose-6-phosphate dehydrogenase (G-6-PDH) has been described[1, 2]. From these investigations a distinct relationship could be established between the inhibitory activity of steroids and their chemical structure. As it appears, the presence of an oxo group in C-17 or C-20 and a 3β-hydroxy group, as well as a planar configuration of rings A and B such as in 5α-, Δ^4- or Δ^5-steroids is required for optimal activity as a non-competitive inhibitor of human G-6-PDH. Of the numerous steroids tested dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one, DHA) proved to be one of the most effective steroids in the enzyme inhibition test. Concerning the activity of steroid conjugates, DHA sulfate and glucuronoside failed to inhibit human G-6-PDH even at relatively high concentrations[3, 4]. On the other hand, the activity of DHA sulfatide greatly exceeded that of the free steroid. Such findings led to the question of whether the enzyme inhibition by the latter conjugate depends in part on its pronounced lipophile properties, which for instance facilitate its penetration of biological membranes[5].

The present communication therefore deals with the inhibition of human placental G-6-PDH by DHA, its conjugates and other lipophile derivatives. At the same time the influence of proteins and lipids upon the activity of DHA, DHA sulfate and DHA sulfatide was examined.

MATERIALS AND METHODS

Placental G-6-PDH was prepared and purified as outlined in a preceding publication[6]. As compared to the specific activity of a crude enzyme preparation of 8·5 mU/mg protein, the purified enzyme exhibited a specific activity of 1120

mU/mg protein, indicating a 132-fold enrichment of the enzyme. All DHA derivatives tested were prepared by known procedures[7–9] and purified by recrystallization and repeated thin layer chromatography in suitable solvent systems.

In the first series of experiments 0·1 ml of the purified enzyme preparation in 0·05 M triethanolamine/0·005 M EDTA buffer of pH 7·6 was diluted with 3·0 ml of the same buffer, eventually containing 40 mg albumin, 8 mg α-globulin, 8 mg β-globulin or 7 mg γ-globulin (SERVA Feinbiochemika, Heidelberg, Germany) per ml. Then 0·1 ml 0·01 M NADP solution, 0·01–0·02 ml dioxane with steroid or steroid derivative and 0·05 ml 0·031 M glucose-6-phosphate solution were added. The final concentration of the steroid or steroid derivative corresponded to a 10^{-5} M or 10^{-6} M solution. When lipids were to be added, 0·01 ml of steroid solution and 0·01 ml 0·003 M lecithin or cholesterol stearate (MERCK AG, Darmstadt, Germany) or 0·01 ml dioxane with plasma lipids (equivalent to 1 ml plasma) were employed. From the changes in the absorption at 366 nm, measured over 10 min at 25°C after a 5-min preincubation period, the enzyme activity was determined and compared to that of the appropriate blank, e.g. enzyme and substrates without steroid.

In the second series the activity of the enzyme was assayed in the presence of constant amounts of steroid or steroid derivative (10^{-5} M), of excess NADP and limiting concentrations of glucose-6-phosphate, the results being evaluated by the methods of Hunter and Downs[10] for estimation of K_i-values.

RESULTS

In Table 1 the % inhibition of placental G-6-PDH by 10^{-5} M and 10^{-6} M concentrations of DHA and its different conjugates is listed. The K_i-values of DHA, its conjugates or derivatives for glucose-6-phosphate as substrate are given in Table 2. The effect of proteins or lipids upon the inhibitory activity of DHA, DHA sulfate and DHA sulfatide can be seen in Table 3.

DISCUSSION

From the results shown in Table 1 it becomes quite apparent that in contrast to free DHA or to DHA sulfatide the steroid sulfate, phosphate or glucuronoside are practically devoid of activity in the G-6-PDH inhibition test. The minor activity observed with DHA sulfate may be attributed to a partial hydrolysis of this conjugate by contaminating steroid sulfatase in the enzyme preparation[6, 11]. Such findings agree with the hypothesis that the negative charges in the anionic

Table 1. Inhibition of human placental G-6-PDH by DHA and its conjugates

Compound	% Inhibition at a concentration of: 10^{-5} M	10^{-6} M
DHA	53	14
DHA sulfate	7	3
DHA phosphate	3	–
DHA glucuronoside	2	–
DHA sulfatide, synthetic[9]	82	30
DHA sulfatide, biosynthetic[12]	80	29
DHA sulfatide, from human plasma[14]	85	33

Table 2. K_i-values for glucose-6-phosphate (G-6-P) of DHA, its conjugates and derivatives

Compound	K_i-value for G-6-P
DHA	$0{\cdot}79 \times 10^{-5}$ M
DHA sulfate	31
DHA phosphate	32
DHA glucuronoside	33
DHA sulfatide, synthetic	0·36
DHA sulfatide, biosynthetic	0·38
DHA sulfatide, from human plasma	0·33
DHA acetate	3·7
DHA caproate	13
DHA laurate	17
DHA stearate	20

Table 3. Effects of proteins or lipids on the inhibition of placental G-6-PDH by DHA or its conjugates

Added	Concentration	% Inhibition at 10^{-5} M concentrations of: DHA	DHA sulfate	DHA sulfatide
Albumin	40 mg/ml	2	5	4
γ-globulin	8	45	4	69
β-globulin	8	42	5	72
γ-globulin	7	48	7	74
Lecithin	10^{-5} M	51	6	76
Cholesterol stearate	10^{-5} M	52	13	84
Plasma lipids	1 ml plasma equiv.	58	6	79
Albumin + lecithin	40 mg/ml 10^{-5} M	3	6	5
Albumin + cholesterol stearate	40 mg/ml 10^{-5} M	2	5	6
Albumin + plasma lipids	40 mg/ml 1 ml plasma equiv.	3	5	6

conjugates prevent their interaction with the enzyme molecule. The activity of DHA sulfatide, whether prepared by chemical synthesis[9], by incubation of DHA sulfate with liver mitochondria[12] or by isolation from human plasma, clearly exceeded that of the free steroid. This is also demonstrated by the K_i-values of the various compounds listed in Table 2. With glucose-6-phosphate as substrate the K_i-values of the different DHA sulfatides resembled each other. Since the molarity of the endogenous DHA sulfatide[13, 14] refers only to the DHA content of the material isolated from plasma, the presence of additional 17-oxo steroids may very well be responsible for a slightly lower K_i-value of this preparation. In view of a distinct, inverse relationship between human red blood cell G-6-PDH and levels of sulfoconjugated DHA in plasma or urine under physiological conditions[15–17] the failure of DHA sulfate to inhibit human G-6-PDH[4] suggests that the endogenous sulfoconjugate of DHA is not identical with DHA sulfate. Instead, the present data as well as previous findings [13–15] support the role of the lipophile DHA sulfatide as the endogenous sulfoconjugate. That the remarkable inhibition of G-6-PDH by free DHA or by

DHA sulfatide does not depend on the lipophile properties of these compounds may be derived from Table 2, where also the K_i-values of several lipophile DHA derivatives are included. The increase in the chain length of esterifying alkanoic acids obviously promotes the lipophile properties of the particular DHA alkylate. At the same time, however, the activity of the compound in the enzyme inhibition test was found to be reduced.

Concerning the effects of proteins or lipids on the activity of DHA, DHA sulfate and DHA sulfatide the addition of lipids like lecithin, cholesterol stearate or endogenous plasma lipids did not improve upon the inactivity of the steroid sulfate. Hence, the formation of heteropolar complexes between the anionic steroid sulfate and quaternary ammonium groups such as in lecithin[18] does not cause the conformational changes in the enzyme molecule which are considered necessary for a non-competitive or allosteric inhibition. Likewise, any interaction between the steroid sulfate and the guanidinium group of arginine in serum albumin[19] failed to induce inhibitory activity. Only the admixture of cholesterol stearate to the incubate resulted in a minor rise of the activity of the steroid sulfate. Whereas the presence of lipids did not affect the inhibition of placental G-6-PDH by DHA or DHA sulfatide, the addition of albumin in physiological concentrations led to an almost complete loss of activity. Whether this interference in the inhibition of G-6-PDH by albumin is solely due to an extensive binding of free steroid[20] or steroid sulfatide[21] remains to be seen. The addition of lipids to albumin containing incubates did not restore the inhibitory capacity of free DHA or DHA sulfatide. On the basis of several *in vivo* studies[15–17], dealing with the influence of sulfoconjugated DHA upon red blood cell G-6-PDH, no such effects of albumin seem to occur under physiological conditions.

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