Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 493–496. Pergamon Press. Printed in Great Britain

THE EFFECTS OF STEROIDS ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE

G. W. OERTEL and P. BENES
Abteilung für Experimentelle Endokrinologie, Universitäts-Frauenklinik, D-65 Mainz, Germany

SUMMARY

The interaction between steroids or steroid conjugates and G-6-PDH, eventually leading to an allosteric inhibition of the enzyme, depends on several structural elements in the steroid molecule. In addition to a 17- or 20-oxo group, an oxygen function at C-3 in a planar ring A/B configuration and probably a certain electron density at C-5 are required for optimum inhibitory activity. Whereas anionic conjugates appear to be devoid of said biological activity, the esterification with sulfatidic acid markedly enhances the inhibition of this enzyme.

INTRODUCTION

For 10 years it has been known that mammalian glucose-6-phosphate dehydrogenase (G-6-PDH) is non-competitively inhibited by numerous steroids. From their initial experiments Marks *et al.*[1] concluded that said inhibition depends solely on the presence of a 17- or 20-oxo group in the steroid molecule. Since, however, the inhibitory activity of 17- or 20-oxo steroids varies considerably from one compound to the next, other structural features must also be involved in steroid-protein interactions.

In order to obtain further information on the interrelationship between the chemical structure of a steroid or steroid conjugate and its biological activity the effects of different C_{19}- and C_{21}-steroids and some derivatives upon human placental G-6-PDH were studied.

MATERIALS AND METHODS

Placental G-6-PDH was purified by a fractionate ammonium sulfate precipitation, adsorption on calcium phosphate gel and a second ammonium sulfate precipitation, resulting in a 115-fold enrichment of the enzyme. The K_M-value of the purified enzyme preparation was found to be $8{\cdot}6 \times 10^{-5}\,M$ for glucose-6-phosphate and $6{\cdot}9 \times 10^{-5}\,M$ for NADP. The inhibition tests were performed in $0{\cdot}05\,M$ triethanolamine/$0{\cdot}005\,M$ EDTA buffer of pH 7·6, using $0{\cdot}01\,M$ NADP and decreasing concentrations of glucose-6-phosphate for substrate. The final concentration of steroidal compounds, added in 0·02 ml dioxane, corresponded to a $10^{-5}\,M$ to $10^{-7}\,M$ solution. From changes in the absorption at 366 nm of samples and appropriate blanks the inhibition was determined and the K_i-values for glucose-6-phosphate calculated by the method of Hunter and Downs[2].

RESULTS AND DISCUSSION

As can be derived from Table 1, of the various 3-substituted 4-ene- or 5-ene-C_{19}-steroids, 3β-hydroxy-5-androsten-17-one (dehydroepiandrosterone, DHA) proved to be the most effective inhibitor of placental G-6-PDH, closely followed by its 4-ene-isomer. The reduction of the 17-oxo group of DHA to the 17β-hydroxy group led to an almost complete loss of activity, stressing the importance of the 17-oxo group. On the other hand, the replacement of the 3β-hydroxy group

Table 1. Inhibition of placental G-6-PDH by unsaturated C_{19}-steroids

Compound	K_i-value for G-6-P
3β-hydroxy-5-androsten-17-one	$0{\cdot}79 \times 10^{-5}$ M
3β-hydroxy-4-androsten-17-one	0·80
3β,16α-dihydroxy-5-androsten-17-one	1·0
3β-hydroxy-5-androstene-16,17-dione	2·0
3β,7β-dihydroxy-5-androsten-17-one	2·3
3β,7α-dihydroxy-5-androsten-17-one	2·9
3β,19-dihydroxy-5-androsten-17-one	3·0
4-androstene-3,17-dione	3·0
3β-chloro-5-androsten-17-one	3·2
3β-hydroxy-5-androstene-7,17-dione	6·0
3α-hydroxy-4-androsten-17-one	6·8
17α-hydroxy-4-androsten-3-one	7·8
3β,17β-dihydroxy-5-androsten-16-one	8·2
17β-hydroxy-4-androsten-3-one	13
4-androstene-3,11,17-trione	23
5-androstene-3β,17β-diol	25
5-androstene-3β,16α,17β-triol	37
11β-hydroxy-4-androstene-3,17-dione	52

Table 2. Inhibition of placental G-6-PDH by 3β-hydroxy-5-ene-C_{21}-steroids

Compound	K_i-value for G-6-P
3β-hydroxy-5-pregnen-20-one	$0{\cdot}77 \times 10^{-5}$ M
3β,21-dihydroxy-5-pregnen-20-one	1·1
3β,17α,21-trihydroxy-5-pregnen-20-one	1·9
3β,17α-dihydroxy-5-pregnen-20-one	2·2
5-pregnene-3β,20α-diol	12
5-pregnene-3β,20β-diol	17
5-pregnene-3β,17α,20β-triol	53

by a 3β-chloro group greatly reduced the inhibitory activity. Furthermore, the introduction of additional functional groups in the DHA molecule significantly decreased the inhibition, especially when these groups were located near C-5. This is evidenced by a distinctly higher K_i-value of 7- or 19-hydroxy-DHA as compared to that of 16α-hydroxy-DHA. Similar results were obtained with 3β-hydroxy-5-ene-C_{21}-steroids, where 3β-hydroxy-5-pregnen-20-one (pregnenolone) was found to be as active in the enzyme inhibition test as DHA (Table 2). Again the reduction of the 20-oxo group eliminated the inhibitory activity, while additional hydroxy groups in the pregnenolone molecule caused a noticeable rise of K_i-values. When saturated C_{19}-steroids were submitted to the enzyme inhibition test (Table 3) the K_i-value of 3β-hydroxy-5α-androstan-17-one (epiandrosterone) reflected an even higher inhibitory activity than that of DHA. Whereas by oxidation of the 3β-hydroxy group to the 3-oxo group the inhibition of the enzyme was reduced to roughly one half, epimerization of the 3β-hydroxy group to the 3α-

hydroxy group of androsterone effected a 70 per cent loss of activity. 17β-Hydroxy-C_{19}-steroids and 17-oxo steroids of the 5β-series turned out to possess only little activity in the enzyme inhibition test.

From such findings it may be concluded that the presence of a 17- or 20-oxo group and an equatorial 3β-hydroxy group in a planar ring A/B configuration provide optimum activity of a free steroid in the enzyme inhibition test. Also a certain role may be attributed to the electron configuration at C-5 of the steroid molecule. At the same time these results hint at a 3-point attachment of the steroid inhibitor to the protein molecule, leading to conformational changes of the latter and consequently to an allosteric inhibition of the enzyme.

Concerning the inhibition of placental G-6-PDH by steroid conjugates, which are known to prevail under *in vivo* conditions, Table 4 reveals that the sulfate,

Table 3. Inhibition of placental G-6-PDH by saturated C_{19}-steroids

Compound	K_i-value for G-6-P
3β-hydroxy-5α-androstan-17-one	$0{\cdot}59 \times 10^{-5}$ M
5α-androstane-3,17-dione	1·8
3α-hydroxy-5α-androstan-17-one	4·0
3β,11β-dihydroxy-5α-androstan-17-one	4·7
3β-hydroxy-5β-androstan-17-one	8·5
5β-androstane-3,17-dione	9·4
5α-androstane-3β,17β-diol	11
3α-hydroxy-5α-androstane-11,17-dione	14
17β-hydroxy-5β-androstan-3-one	14
5β-androstane-3,11,17-trione	20
5β-androstane-3β,17β-diol	22
3α-11β-dihydroxy-5α-androstan-17-one	27
3α-hydroxy-5β-androstan-17-one	33
3α-hydroxy-5β-androstane-11,17-dione	38
5β-androstane-3α,17β-diol	46
3α,11β-dihydroxy-5β-androstan-17-one	82

Table 4. Inhibition of placental G-6-PDH by steroid conjugates*

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

*From Benes, P. and Oertel, G. W.: *J. Steroid Biochem.* 2 (1971) 289.

phosphate and glucuronoside of DHA as one of the most active free steroids do not influence the oxidation of glucose-6-phosphate. Minor inhibitory effects of DHA sulfate obviously are due to a partial hydrolysis of the conjugate by contaminating placental sulfatase[3, 4]. Conversely, DHA sulfatide – the DHA ester of sulfatidic acid – exhibited an outstanding inhibotory activity. The K_i-value of the synthetic DHA saulfatide approximated that of the biosynthetic sulfatide, prepared by incubation of liver mitochondria with DHA sulfate, as well as that of endogenous DHA sulfatide from peripheral human plasma. At a 10^{-7} *M* concentration all 3 DHA sulfatides still produced a 15–20 per cent inhibition of placental G-6-PDH. That the biological activity is not related to the lipophile properties of the steroid or steroid conjugate may be seen from the lack of activity of DHA alkylates. An increase in the chain length of the esterifying alkanoic acid, accentuating the lipophile properties, actually decreased the inhibitory activity of the derivative. Hence, the polarity of the oxygen at C-3 of the steroid molecule appears to be equally important for the assumed interaction between the inhibitor and the enzyme molecule.

REFERENCES

1. P. A. Marks and J. Banks: *Proc. Natn. Acad. Sci. (U.S.A.)* **46** (1961) 10.
2. A. Hunter and C. E. Downs: *J. biol. Chem.* **157** (1945) 427.
3. A. P. French and J. C. Warren: *Steroids* **6** (1965) 865.
4. P. Menzel, M. Gobbert and G. W. Oertel: *Hormone Metab. Res.* **2** (1970) 225.

DISCUSSION

Fazekas (A. G.): Dr. Oertel, I think your results are extremely interesting especially concerning the DHA-sulfatide. If we consider the concentration of DHA-sulfate in the human blood as 100 μg/100 ml then what is the proportion of DHA-sulfatide in this?

Oertel: We think that the ratio of steroid sulfatide to steroid sulfate is probably 9 : 1 in favour of the lipophile sulfoconjugate.

Fazekas (A. G.): This I find very important, because in this case the glucose-6-phosphate dehydrogenase of the hair follicles is inhibited approximately 60–80% at physiological concentrations of the DHA-sulfatide.

Oertel: Do you not mean sulphate.

Fazekas (A. G.): No sulfatide. If you say that 90% of the circulating DHA-sulfate is present in the form of sulfatide, then the enzyme is inhibited 60–80%. In other words, it is physiologically regulated by DHA-sulfatide.

Oertel: We also think that the steroid sulfate is unable to penetrate biological membranes because after incubation of washed erythrocytes or whole blood with labeled dehydroepiandrosterone sulfate no radioactivity was found within the erythrocytes. This also holds for similar experiments with mitochondria. Such findings are certainly not compatible with the generally accepted hypothesis that the steroid sulfate exists as such in the human organism. The existence of steroid sulfatide, moreover, allows for the regulation of glucose-6-phosphate dehydrogenase, the interrelationship between sulfoconjugated dehydroepiandrosterone and this enzyme having been established by various investigators under in-vivo conditions.

Liao: I wonder if you've tried estrogens, especially estrone.

Oertel: We are just doing this.

Liao: I asked this because the geometry of the 5-en compound is more similar to estrogen than androgen.